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(71) Applicant *(for all designated States except US)*: **CANCER RESEARCH VENTURES LIMITED** [GB/GB]; 5 Alfred Place, London, Greater London WC1E 7EB (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HADFIELD, John, Anthony** [GB/GB]; c/o The University of Salford, Salford, Greater Manchester M5 4WT (GB). **MCGOWN, Alan, Thomson** [GB/GB]; c/o Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, Greater Manchester M20 4BX (GB). **MAYALARP, Stephen, Patrick** [GB/GB]; c/o Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, Greater Manchester M20 4BX (GB). **LAND, Edward, John** [GB/GB]; c/o Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, Greater Manchester M20 4BX (GB). **HAMBLETT, Ian** [GB/GB]; c/o Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, Greater Manchester M20 4BX (GB). **GAUKROGER, Keira** [GB/GB]; c/o Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, Greater Manchester M20 4BX (GB). **LAWRENCE, Nicholas, James** [GB/GB]; c/o Department of Chemistry, Cardiff University, PO Box 912, Cardiff CF10 3TB (GB). **HEPWORTH, Lucy, Annette** [GB/GB]; c/o Department of Chemistry, Umist, PO Box 88, Manchester, Greater Manchester M60 1QD (GB). **BUTLER, John** [GB/GB]; c/o Department of Biological Sciences, Salford University, The Crescent, Salford, Greater Manchester M5 4WT (GB).

(74) Agents: **KIDDLE, Simon, J.** et al.; Mewburn Ellis, York House, 23 Kingsway, London, Greater London WC2B 6HP (GB).

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(54) Title: SUBSTITUTED STILBENES AND THEIR REACTIONS

(57) Abstract: The present invention relates to stilbene and quinone compounds related to combretastatin A-4 and their use as anticancer compounds and prodrugs. The compounds include those with an alkyl group on the double bond of cis or trans-stilbenes, compounds with one or more (and preferably 2 or 3) alkyl group substituents on the stilbene A ring, compounds with an alkoxy group other than methoxy at position 3, 4, and/or 5 of the stilbene A ring, compounds (or prodrugs) in which BOC amino acid esters are formed with the phenolic hydroxyl at the 3-position of the B ring and compounds (or prodrugs) based on a benzoquinone B ring. The present invention further relates to the photochemical reactions of stilbene compounds, either the above compounds disclosed for the first time herein or compounds based on prior art stilbenes. These reactions include the photochemical release of an active form of the compound from a prodrug conjugate and the photochemical isomerisation of the compounds, especially from a trans to cis form of compounds. The reactions can be used alone or in combination to convert inactive or comparatively less active forms of the compounds to more active forms,thereby allowing the compounds to be selectively targeted, e.g. activating them at the site of a tumour.

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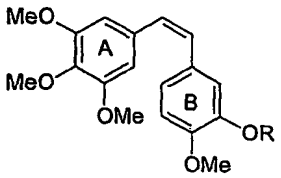
Substituted Stilbenes and Their Reactions

Field of the Invention

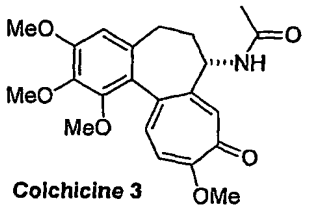
The present invention relates to novel compounds, and more particularly to stilbene and quinone compounds related to combretastatin A-4 and their possible use as anticancer compounds and prodrugs. In further aspects, the present invention relates to the photochemical reactions of some of these compounds, in the photochemical isomerisation of the compounds and/or the photochemical release of an active compound from a protected compound (prodrug).

Background of the Invention

The stilbene *cis*-combretastatin A-4 *Z*-**1**, isolated from the African bush willow, *Combretum caffrum* shows exciting potential as an anticancer agent, binding strongly to tubulin and displaying potent and selective toxicity toward tumour vasculature (US Patent No:4,996,237, Arizona Board of Regents, Pettit et al, *Experimentia*, 1989, *45*, 209; Lin et al, *Mol. Pharmacol.*, 1988, *34*, 200; Grosios et al, *Brit. J. Cancer*, 1999, *81*, 1318; Lin et al, *Biochemistry*, 1989, *28*, 6984; Woods et al, *Brit. J. Cancer*, 1995, *71*, 705; McGown et al, *Cancer Chemother. Pharmacol.*, 1990, *26*, 79; El-Zayat et al, *Anti-Cancer Drugs*, 1993, *4*, 19; Dark et al, *Cancer Research*, 1997, *57*, 1829.).



Z-**1**, R = H
Z-**2**, R = $P(O)(ONa)_2$



Colchicine 3

Cis-combretastatin A-4 *Z*-**1** is able to inhibit cell growth at low concentrations (IC_{50}, P388 murine leukaemia cell line 2.6 nM). The potency of *trans*-combretastatin A-4 *E*-**1** is much lower and inhibits cell growth in the μM range. Arguably, it is the ability of *Z*-**1** and *Z*-**2** to destroy tumour blood vessels, effectively starving tumours of

nutrients, which makes them such exciting molecules. Tumour vasculature and the formation of neovasculature were first identified as a target for cancer therapy by Judah Folkman some 30 years ago. The work of Folkman and others has clearly identified angiogenesis and blood supply as necessary requirements for primary tumour growth, invasiveness and metastasis. It is now becoming clear that the selective destruction of tumour vasculature will have a significant impact on the clinical treatment of cancer. Angiogenesis is subject to a complex process of regulation and thereby offers a multitude of molecular targets for drug design.

The use of *Z*-**1** as a clinically useful anticancer agent has been severely hampered by its poor water solubility (Brown et al, *J. Chem. Soc., Perkin Trans. 1*, 1995, 577). The phosphate salt *Z*-**2** is more soluble in water than *Z*-**1** and is soon to enter phase II clinical trials (Pettit et al, *Anti-Cancer Drug Des.*, 1995, *10*, 299). Nevertheless, both *Z*-**1** and *Z*-**2** are not targeted towards cancer cells and their therapeutic efficacy would be improved if their selectivity were better. The low solubility of *cis*-combretastatin A-4 in water and saline has led to attempts in the art to make related compounds or prodrugs which retain the activity of *cis*-combretastatin A-4 as an anticancer agent and which have enhanced solubility. These attempts focus on forming salts or derivatives at the phenolic hydroxyl group of combretastatin. By way of example, US Patent No: 5,561,122 (Arizona Board of Regents) discloses the sodium and potassium salts of *cis*-combretastatin A-4 and a hemisuccinic acid ester derivative, and WO99/35150 (Arizona Board of Regents) discloses the lithium, caesium, magnesium, calcium, manganese and zinc salts of *cis*-combretastatin A-4, and ammonium cation salts with imidazole, morpholine, piperazine, piperidine, pyrazole, pyridine, adenosine, cinchonine, glucosamine, quinine, quinidine, tetracycline and verapamil.

At the molecular level, both compounds target tubulin, binding strongly at or close to the colchicine (**3**) binding site, preventing polymerisation of α,β-tubulin heterodimer to microtubules. Their inhibition of microtubule formation prevents mitosis and is important in disrupting the growth of new vascular epithelial cells. In addition, disruption of the intracellular microtubule networks by combretastatin A4 leads to the

destruction of microvessels within the tumour. This antivascular activity offers exciting therapeutic possibilities as the destruction of microvessels results in the death of all tumour cells which depend on the vessel for nutrients and oxygen. The multi-functional role of tubulin in both healthy and cancer cells highlights the need for selectively targeted drugs.

We have previously investigated the tubulin-binding properties of agents related to *Z*-**1** and **3** and as part of this effort, we have designed many related compounds that behave in a similar fashion to *Z*-**1** (Ducki et al, *Bioorg. Med. Chem. Lett.*, 1998, 8, 1051; Zhao et al, *Eur. J. Nuc. Medicine*, 1999, *26*, 231; Aleksandrzak et al, *Anti-Cancer Drugs*, 1998, *9*, 545). However, it remains a problem in the art in designing effective compounds and especially those which can be selectively targeted.

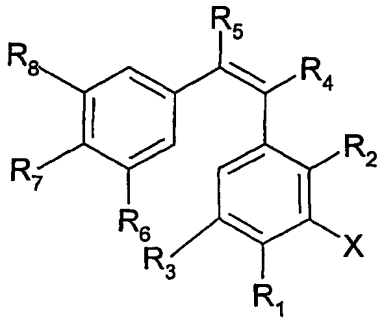
Summary of the Invention

In a first group of aspects, the present invention relates to novel compounds and more particularly to stilbene and quinone compounds related to combretastatin A-4. The synthesis of new compounds is disclosed herein, together with experiments demonstrating their activity *in vitro* and *in vivo*, supporting their use as anticancer compounds and prodrugs. The compounds include those with an alkyl group on the double bond of *cis* or *trans*-stilbenes, compounds with one or more (and preferably 2 or 3) alkyl group substituents on the stilbene A ring, compounds with an alkoxy group other than methoxy at position 3, 4, and/or 5 of the stilbene A ring, compounds (or prodrugs) in which BOC amino acid esters are formed with the phenolic hydroxyl at the 3-position of the B ring and compounds (or prodrugs) based on a benzoquinone B ring.

In a further group of aspects, the present invention relates to the photochemical reactions of stilbene compounds, either the above compounds disclosed for the first time herein or compounds based on prior art stilbenes. These reactions include the photochemical release of an active form of the compound from a prodrug conjugate and the photochemical isomerisation of the compounds, especially from a *trans* to *cis*

form of compounds. The reactions can be used alone or in combination to convert inactive or comparatively less active forms of the compounds to more active forms, thereby allowing the compounds to be selectively targeted, e.g. activating them at the site of a tumour.

Accordingly, in a first aspect, the present invention provides a compound represented by the structural formula:



wherein:

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy;

or a salt or derivative thereof.

In all aspect of the invention, preferably, the substituents are chosen according to the following lists of preferred groups.

Preferably, alkyl or alkoxy substituents are substituted or unsubstituted C_{1-10} alkyl or alkoxy groups. In either case, the alkyl chain can be straight chain or branched.

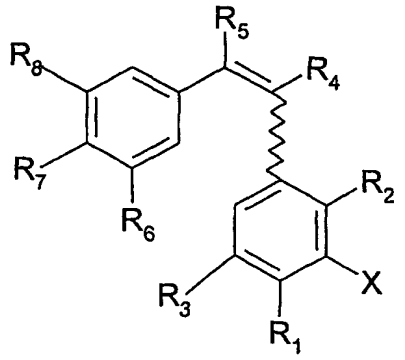
Preferred alkyl substituents are methyl or ethyl. Preferred alkoxy substituents are methoxy or ethoxy.

Halogen substituents can be fluorine, chlorine, bromine or iodine, and are preferably fluorine. Preferably, the haloalkyl groups are fluoroalkyl, and most preferably is a CF_3 group.

Preferably, the O-ester group is represented by the formula O-phosphate, OCO-alkyl, OCO-aryl, OCO-heteroaryl, OCO-amino acid, OCO-peptide, OCO-polymer, OCO-sugar or OCO-CHR-NH-BOC, where BOC represents a t-butoxycarbonyl group.

As used herein, preferably R and R' are substituted or unsubstituted C_{1-10} alkyl groups. R" is preferably selected from substituted or unsubstituted alkyl (e.g.C_{1-10}), aryl or heteroaryl groups.

In a further aspect, the present invention provides compounds in which there are one or more alkyl groups present on the double bond linking the stilbene A and B rings. Thus, in this aspect, the present invention provides compounds represented by the structural formula:



wherein:

the zigzag line indicates that the compound can be *cis* or *trans*;

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-

aryl, O-heteroaryl or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', S, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

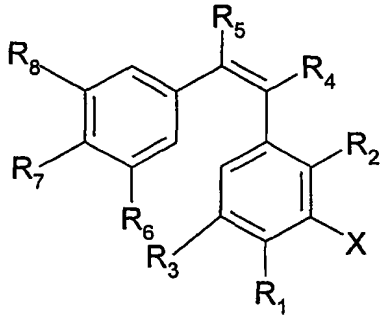
R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy;

wherein at least one of the substituents R_4 and R_5 is an alkyl group.

or a salt or derivative thereof.

As defined above, the compounds in this aspect of the invention may be either the *cis* or *Z*-isomer, i.e. be related to combretastatin A4, or the *trans* or *E*-isomer. Examples of the synthesis of both isomers are proved below. Preferably, the alkyl group R_4 and/or R_5 is a methyl or ethyl group.

In a further aspect, the present invention provides compounds in which one or more of the methoxy groups on the A ring of combretastatin is replaced by an alkyl group. Thus, in this aspect, the present invention provides compounds represented by the structural formula:



wherein:

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

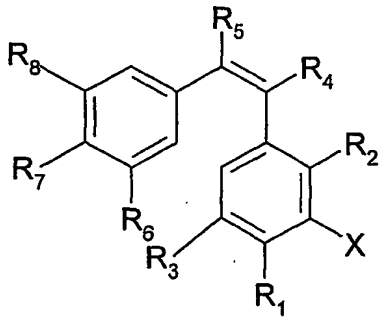
R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

wherein R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy such that at least one of these substituents is an alkyl group;

or a salt or derivative thereof.

In preferred embodiment, two or more preferably all three of the groups are alkyl groups. Exemplary compounds include those with methyl, ethyl or propyl groups. In a preferred embodiment, R_6, R_7 and R_8 are methyl groups.

In a further aspect, the present invention provides compounds in which one or more of the methoxy groups on the A ring of combretastatin is replaced by a higher alkoxy group, i.e. an ethoxy or longer chain group. Thus, in this aspect, the present invention provides compounds represented by the structural formula:



wherein:

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl, or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or

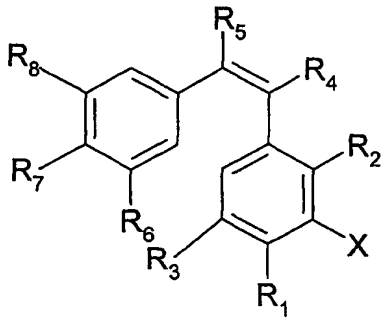
CH_2CONHR"; and,

wherein R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy such that at least one of these substituents is an alkoxy group other than methoxy group;

or a salt or derivative thereof.

Preferably, two or all three of the groups is replaced by an alkoxy group other than methoxy.

In a further aspect, the present invention relates to compounds in which the phenolic hydroxyl group on the B ring of the combretastatin is derivatised to form a *t*-BOC-amino acid ester. These compounds may be prodrugs capable of releasing combretastatin, or a variant thereof, e.g by the action of an enzyme capable of hydrolysing the BOC-amino acid ester, e.g. an esterase enzyme. Thus, in this aspect, the present invention provides compounds represented by the structural formula:



wherein:

R_1 is selected from alkyl, alkoxy, NH_2, NHR, NRR', SR, CF_3, CHO or halogen;

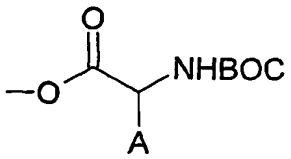
R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy; and,

or a salt or derivative thereof;

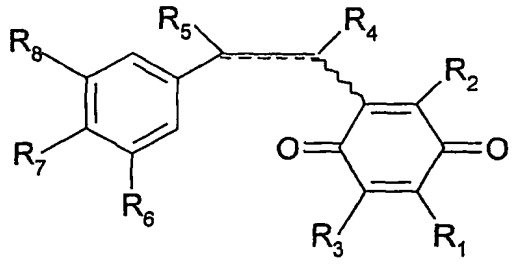
wherein X is a group represented by:



wherein BOC represents a t-butoxycarbonyl group and the A group is an amino acid side chain.

The BOC amino acid ester may include a naturally occurring or synthetic amino acid, in either the D or L-isoform. Examples of compounds of the aspect of the invention include those where the amino acid is Phe, Ile, Gly, Trp, Met, Leu, Ala, His, Pro, D-Met, D-Trp, or Tyr, e.g. when in compound 33 the amino acid is Phe, the A group is - CH_2Ph etc.

In a further aspect, the present invention provides compounds in which the B ring of combretastatin is replaced by a substituted or unsubstituted benzoquinone ring. These quinone compounds may act as prodrugs of combretastatin and be activated *in vivo* by enzymes such as DT-diaphorase. Thus, in this aspect, the present invention provides compounds represented by the structural formula:



wherein:

the dotted line indicates a single or double covalent bond and the zigzag line indicates that the compound can be *cis* or *trans*;

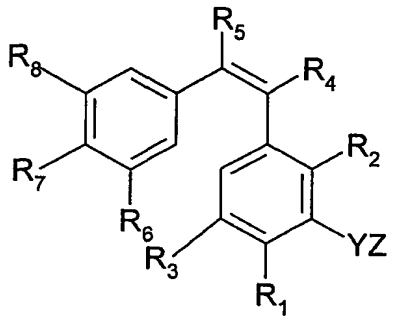
R_1, R_2 and R_3 are independently selected from hydrogen, alkyl, CHO, COR, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy; or a salt or derivative thereof.

The present invention also includes compositions comprising one or more of the above defined compounds. In other aspects, the present invention provides the compounds for use in a method of medical treatment and the use of the compounds for the preparation of a medicament for the treatment of a condition that responds to the medicament, and in particular for the treatment of cancer. The compounds may act directly or be prodrugs capable of releasing an active form of the compound upon hydrolysis or reduction, e.g. as mediated *in situ* by an enzyme.

In the second group of aspects, the present invention provides a prodrug comprising a compound conjugated to a photocleavable group, wherein the prodrug is represented by the general formula:



wherein:

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR'' or CH_2CONHR''; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkyoxy;

Y is selected from O, S, Se, NH; and,

Z is a photocleavable group;

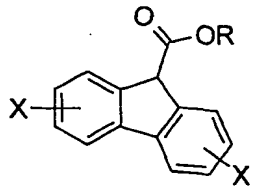
or a salt or derivative thereof.

The compounds conjugated to the photocleavable group to form the prodrug may be the new combretastatin derivatives disclosed herein or may be a known combretastatin which has not be conjugated in this way in the prior art.

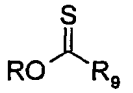
The prodrugs can be activated by exposure to electromagnetic radiation, especially ultraviolet-visible light (e.g. having a wavelength of between about 190-1000nm), to remove the protecting photocleavable group and cause the release of the compound. Thus, the prodrugs can be used to provide selective activation of the active form of the compound, e.g. at the site of a tumour, by administering the compound and exposing to light the site at which activation is required.

Particularly preferred compounds (prodrugs) are those which can be exposed to light to release combretastatin, and especially *cis*-combretastatin A4.

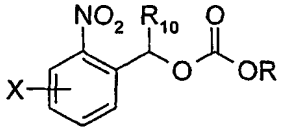
Examples of preferred compounds include those which Z, the photocleavable group is selected from:



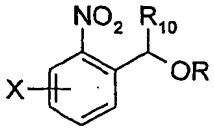
D. H. R Barton, Y. L. Chow, A. Cox, and G. W. Kirby, *J. Chem. Soc.*, **1965**, 3571.



T. Kishi, T. Tsuchiya and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **1979**, 52, 3015.



L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, **1978**, *100*, 8006.



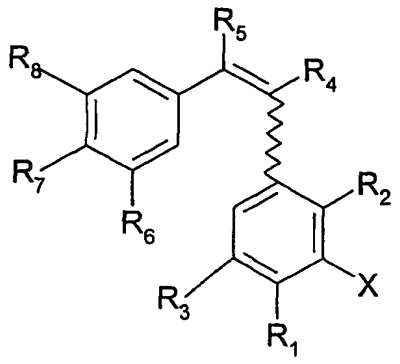
Patchornik, A.; Amit, B.; Woodward R. B. *J. Am. Chem. Soc.*, **1970**, 92, 6333-6335
B. Amit, E. Hazum, M. Fridkin, and A. Patchornik, *Int. J. Pept. Protein Res.*, **1977**, *9*, 91.

In the above formulae, R is the photoprotected group, R_9 and R_{10} are independently selected from alkyl, aryl or heteroaryl and X is any functional group. Examples of photoactivatable groups are also provided on pages 54-59.

In a further aspect, the present invention provides the compounds as defined herein for use in a method of medical treatment. In preferred embodiments, the present invention provides the use of the compounds defined herein for the preparation of a medicament for the treatment of a condition that is ameliorated by administration of the activated or released form of the compound. In such uses, it is preferred that the activated form of the compound has significantly greater activity than the protected form of the compound, e.g. making it possible to obtain selectivity in the delivery and activation of the compound, e.g. to a target tissue. In preferred embodiments of the invention, the compounds are employed in medicaments for the treatment of cancer.

In a further aspect, the present invention provides a process for providing the compound at a site, the process comprising exposing a prodrug represented by the above formula to light to release the compound at the site. In this embodiment of the invention, preferably the light is in the visible range, e.g. from about 350-800nm.

In a further aspect, the present invention provides a process for isomerising a compound represented by the general formula:



wherein:

R_1 is selected from alkyl, alkoxy, CHO, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR'' or CH_2CONHR''; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy;

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl, O-ester, or the group Y-Z as defined above;

or a salt or derivative thereof;

the process comprising exposing the compound to light so that it isomerises from the *E*-isomer to the *Z*-isomer. This process might be carried out separately or in conjunction with the light activated release of the compound from a prodrug as defined above.

In a further aspect, the present invention provides a process for producing the photoactivatable compounds defined herein, the process comprising linking a

photoactivatable group to the Y group of a precursor compound to produce photoactivatable compounds as defined above.

The work disclosed herein arises from the findings that the inactive *trans* isomer of combretastatin A-4 *E*-**1** can be converted to the active *cis*-isomer *Z*-**1** by the action of ultraviolet light *ex situ* in a photochemical reactor. The irradiation of *E*-**1** in this manner leads to an impressive and rapid increase in activity. Further we have found that only after a long period of irradiation is the formation of the phenanthrene (which is only moderately active, as measured by its ability to inhibit cancer cell growth *in vitro*, IC_{50} 0.7 μM) evident. We have prepared phenanthrene, by irradiation of *E*-**1**, in good yield when an oxidant (I_2) is present (to oxidize the first-formed cyclization product). This provides an opportunity to exploit the hypoxic nature of solid tumours and increase the selectivity of irradiated *E*-**1**. In other words, healthy cells may provide an oxidative pathway for the formation of the less toxic phenanthrene and effectively decrease the lifetime of *Z*-**1**.

The same result can be achieved *in situ* in the presence of cultured cancer cells (K562 human myelogeneous leukaemia cell line. These experiments showed that within 2 seconds of exposure to ultraviolet light the activity of the *E*-combretastatin A-4 (IC_{50} originally 5 μM) increases to 2 nM, providing a rapid thousand-fold increase in activity. Moreover, the cells in the absence of the drug are not affected by exposure to the radiation and grow normally over the 5 days of the assay. To increase the water solubility of the drug we have produced prodrugs with a photo-cleavable group attached to the B-ring phenolic OH group. The nitro vanillin derivative was chosen since it has been used as a photo-cleavable linker for solid phase synthesis applications and its synthesis is relatively simple. These prodrugs have been successfully cleaved in both the *E* and *Z* series (*E*-**6** and *Z*-**6** respectively) and have produced highly cytotoxic agents *in vitro* upon *in situ* exposure to ultra violet radiation. The cleavage of the water solubilising group appears to be faster than the *E*→*Z* isomerisation, at least under *ex situ* irradiation. Thus, the use of *Z*-**6** has some merit. Indeed it forms the prototype for systems that do not rely on any special photochemical features of the molecule to be delivered. This provides a more general approach to the site-specific photochemical activation of prodrugs. Moreover, the photocleavable water solubilising group can be engineered, so that cleavage occurs at longer wavelength and rapidly.

Embodiments of the present invention will now be described by way of example and not limitation with reference to the accompanying figures.

Brief Description of the Figures

Figure 1 shows the anti-tumour activity of compound 97-64H in an *in vivo* tumour implant experiment in mice.

Figure 2 shows the anti-tumour activity of compound 97-96 in an *in vivo* tumour implant experiment in mice.

Detailed Description

Pharmaceutical Compositions

The compounds of the invention may be derivatised in various ways. As used herein "derivatives" of the compounds includes salts, esters such as *in vivo* hydrolysable esters, free acids or bases, hydrates, prodrugs or coupling partners. In the case of compounds which are combretastatin or analogues thereof, preferably the derivatives are soluble in water and/or saline or can be hydrolysed to provide physiologically active agents.

Examples in the prior art of salts or prodrugs of *cis*-combretastatin A-4 focus on forming salts or derivatives at the phenolic hydroxyl group of combretastatin. These include sodium phosphate salts, sodium and potassium salts (US Patent No: 5,561,122), lithium, caesium, magnesium, calcium, manganese and zinc salts of *cis*-combretastatin A-4, and ammonium cation salts with imidazole, morpholine, piperazine, piperidine, pyrazole, pyridine, adenosine, cinchonine, glucosamine, quinine, quinidine, tetracycline and verapamil (WO99/35150).

Salts of the compounds of the invention are preferably physiologically well tolerated and non toxic. Many examples of salts are known to those skilled in the art. Compounds having acidic groups, can form salts with alkaline or alkaline earth metals such as Na, K, Mg and Ca, and with organic amines such as triethylamine and Tris (2-hydroxyethyl)amine. Salts can be formed between compounds with basic groups, e.g. amines, with inorganic acids such as hydrochloric acid, phosphoric acid or sulfuric acid, or organic acids such as acetic acid, citric acid, benzoic acid, fumaric acid, or tartaric acid. Compounds having both acidic and basic groups can form internal salts.

Esters can be formed between hydroxyl or carboxylic acid groups present in the compound and an appropriate carboxylic acid or alcohol reaction partner, using techniques well known in the art. Examples of esters include those formed between the phenolic hydroxyl of the substituted stilbenes and carboxylic acids, hemisuccinic acid esters, phosphate esters, BOC esters, sulphate esters and selenate esters.

Derivatives which as prodrugs of the compounds are convertible *in vivo* or *in vitro* into one of the parent compounds. Typically, at least one of the biological activities of compound will be reduced in the prodrug form of the compound, and can be activated by conversion of the prodrug to release the compound or a metabolite of it. Example of prodrugs include combretastatin A1 phosphate, combretastatin A4 phosphate and RH1.

Other derivatives include coupling partners of the compounds in which the compounds is linked to a coupling partner, e.g. by being chemically coupled to the compound or physically associated with it. Examples of coupling partners include a label or reporter molecule, a supporting substrate, a carrier or transport molecule, an effector, a drug, an antibody or an inhibitor. Coupling partners can be covalently linked to compounds of the invention via an appropriate functional group on the compound such as a hydroxyl group, a carboxyl group or an amino group.

The compounds described herein or their derivatives can be formulated in pharmaceutical compositions, and administered to patients in a variety of forms, in particular to treat conditions which are ameliorated by the activation of the compound.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder, cream, liquid form or encapsulated by liposomes. A tablet may include a solid carrier such as gelatin or an adjuvant or an inert diluent. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. Such compositions and preparations generally contain at least 0.1wt% of the compound.

Parental administration includes administration by the following routes: intravenous,

cutaneous or subcutaneous, nasal, intramuscular, intraocular, transepithelial, intraperitoneal and topical (including dermal, ocular, rectal, nasal, inhalation and aerosol), and rectal systemic routes. For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, solutions of the compounds or a derivative thereof, e.g. in physiological saline, a dispersion prepared with glycerol, liquid polyethylene glycol or oils.

In addition to one or more of the compounds, optionally in combination with other active ingredient, the compositions can comprise one or more of a pharmaceutically acceptable excipient, carrier, buffer, stabiliser, isotonicizing agent, preservative or anti-oxidant or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. orally or parentally.

Liquid pharmaceutical compositions are typically formulated to have a pH between about 3.0 and 9.0, more preferably between about 4.5 and 8.5 and still more preferably between about 5.0 and 8.0. The pH of a composition can be maintained by the use of a buffer such as acetate, citrate, phosphate, succinate, Tris or histidine, typically employed in the range from about 1 mM to 50 mM. The pH of compositions can otherwise be adjusted by using physiologically acceptable acids or bases.

Preservatives are generally included in pharmaceutical compositions to retard microbial growth, extending the shelf life of the compositions and allowing multiple use packaging. Examples of preservatives include phenol, meta-cresol, benzyl alcohol, para-hydroxybenzoic acid and its esters, methyl paraben, propyl paraben, benzalconium chloride and benzethonium chloride. Preservatives are typically employed in the range of about 0.1 to 1.0 % (w/v).

Preferably, the pharmaceutically compositions are given to an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to

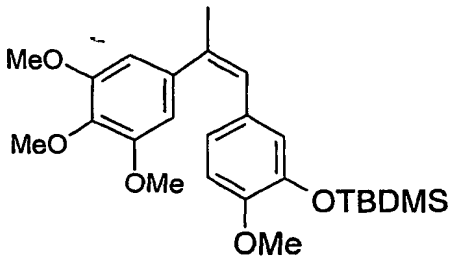
show benefit to the individual. Typically, this will be to cause a therapeutically useful activity providing benefit to the individual. The actual amount of the compounds administered, and rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980. By way of example, and the compositions are preferably administered to patients in dosages of between about 0.01 and 100mg of active compound per kg of body weight, and more preferably between about 0.5 and 10mg/kg of body weight . The compounds may be used in the treatment of cancer and other conditions involving abnormal proliferation of vasculature including diabetic retinopathy, psoriasis and endometriosis.

General

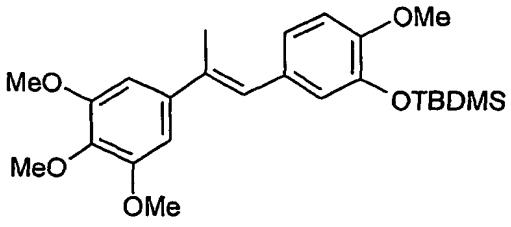
Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Brüker AC 300 (300 MHz) or AC 400 (400 MHz) NMR spectrometer. Chemical shifts, δ, for all NMR spectra are given in ppm, relative to tetramethylsilane, and, unless otherwise stated, using $CDCl_3$ as both solvent and internal standard. Coupling constants (*J*) were measured in Hz. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The UV/VIS spectra were determined using a Hewlett-Packard HP8452 diode-array spectrophotometer. Extinction coefficients (ε) are presented as their natural logarithms. Microanalyses were carried out by the laboratories of the Departments of Chemistry of the University of Manchester and UMIST. High resolution mass spectroscopy was determined using a Kratos Concept 15 mass spectrometer. Thin layer chromatography (tlc) was performed using precoated aluminium-backed silica gel plates (60 F_{254}) with 0.2 mm thickness (Merck), with observation under UV when necessary. Gas chromatography was carried out using an SE 54 column at 195-225 kPa at 1.5kPa/min. The oven temperature was 180-280°C at 5°C/min.

Example 1: Synthesis of combretastatins with alkyl groups on the double bond

***Z*- and *E*-1-(3'-t-Butyldimethylsilyloxy-4'-methoxyphenyl)-2-(3'',4'',5''-trimethoxyphenyl)propene, 17a, 17b**



17a



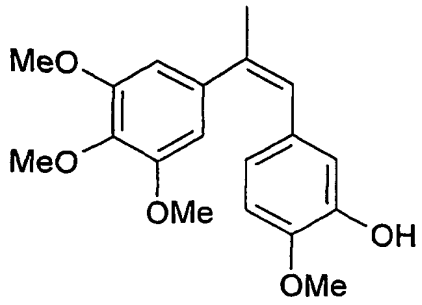
17b

To a slurry of 3-*t*-butyldimethylsilyloxy-4-methoxybenzylphosphonium bromide, **18**, (1 g, 1.69 mmol) in THF (10 ml) was added *n*-butyllithium (1.16 ml of 1.6 M solution, 1.86 mmol) at –15°C. The red anion was stirred for 20 minutes and 3,4,5-trimethoxyacetophenone (355 mg, 1.69 mmol) added. The resultant solution was stirred at room temperature for 1 hour and water (10 ml) carefully added. The aqueous layer was separated and extracted with ether (3 x 10 ml). The combined organic layers were washed with water (2 x 10 ml) and brine (10 ml), dried ($MgSO_4$) and concentrated *in vacuo*.

Following flash column chromatography (SiO_2 petrol:EtOAc 19:1) the *Z* stilbene, **17a**, was isolated as a colourless oil (109 mg, 15%). $R_f = 0.72$ (SiO_2 petrol:EtOAc 1:1); δ_H (300 MHz) 0.20 [6 H, s, $(CH_3)_2$], 1.03 [9 H, s, $(CH_3)_3$], 2.28 (3 H, d, $J = 1.1$, CH_3), 3.85 (3 H, s, OCH_3), 3.89 (3 H, s, OCH_3), 3.93 [6 H, s, $(OCH_3)_2$], 6.69 (1 H, q, J = 1.1, olefinic H), 6.72 (2 H, s, H-2',6') 6.87 (1 H, d, $J = 8.3$, H-5''), 6.91 (1 H, d, J = 2.3, H-2''), 6.95 (1 H, dd, $J = 8.3$, 2.3, H-6''); λ_{max} (MeOH) = 270 (ε = 7,607); M^+, found 444.2329; $C_{25}H_{36}O_5Si$ requires M^+ 444.2332.

Further elution afforded the *E* stilbene, **17b**, as a colourless oil (258 mg, 34%). R_f = 0.67 (SiO_2 petrol:EtOAc 1:1); δ_H (300 MHz) 0.01 [6 H, s, $(CH_3)_2$], 0.92 [9 H, s, $(CH_3)_3$], 2.17 (3 H, d, $J = 1.5$, CH_3), 3.75 [6 H, s, $(OCH_3)_2$], 3.76 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 6.35 (1 H, q, $J = 1.5$, olefinic H), 6.42 (2 H, s, H-2',6') 6.52 (1 H, d, J = 1.9, H-2''), 6.62 (1 H, dd, $J = 8.3$, 1.9, H-6''), 6.67 (1 H, d, $J = 8.3$, H-5''); λ_{max} (MeOH) = 296 (e = 16,541). M^+, found 444.2333; $C_{25}H_{36}O_5Si$ requires M^+ 444.2332.

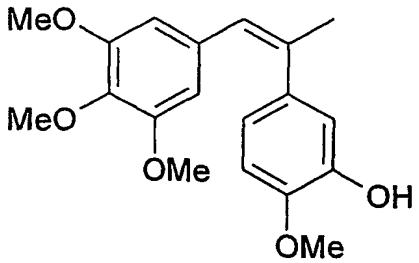
***Z*- (3'-Hydroxy-4'-methoxyphenyl)-2-(3'',4'',5''-trimethoxyphenyl)propene, 19**



19

To a stirred solution of *Z*-(3'-*t*-butyldimethylsilyloxy-4'-methoxyphenyl)-2-(3'',4'',5''-trimethoxyphenyl)propene, **17a**, (111 mg, 0.250 mmol) in dry THF (5 ml) was added tetra-*n*-butylammonium fluoride (700 ml of 1 M solution, 0.7 mmol). The resulting yellow solution was stirred for 20 minutes and then treated with water (2 ml). The aqueous layer was separated and extracted with chloroform (3 x 10 ml). The combined organic layers were washed with water (2 x 10 ml) and brine (10 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Flash column chromatography (SiO_2 petrol:EtOAc 2:1) afforded *Z*-(3'-hydroxy-4'-methoxyphenyl)-2-(3'',4'',5''-trimethoxyphenyl)propene, **19**, as a fine white powder (49 mg, 0.148 mmol, 60%). m.p. 156-8 °C; R_f = 0.36 (SiO_2 petrol:EtOAc 2:1); δ_H (300 MHz) 2.18 (3 H, d, J = 1.5, CH_3), 3.75 [6 H, s, $(OCH_3)_2$], 3.84 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 5.42 (1 H, s, OH), 6.36 (1 H, q, J = 1.5, olefinic H), 6.43 (2 H, s, H-2',6') 6.48 (1 H, dd, J = 8.3, 2.3, H-6''), 6.62 (1 H, d, J = 8.3, H-5''), 6.63 (1 H, d, J = 2.3, H-2''); λ_{max} (MeOH) = 270 (e = 11,524); M^+, found 330.1469; $C_{19}H_{22}O_5$ requires M^+ 330.1467.

Z-1-(3',4',5'-trimethoxyphenyl)-2-(3''-hydroxy-4''-methoxyphenyl)propene, 20

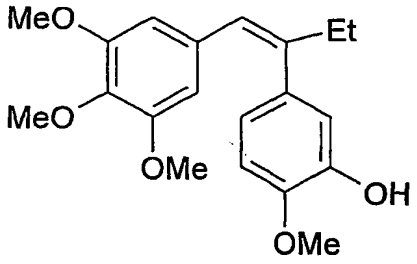


20

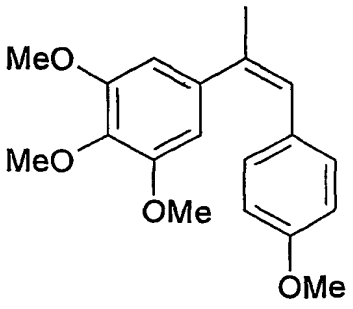
A mixture of *cis*-1-(3',4',5'-trimethoxyphenyl)propene **21** (0.42 g, 2 mmol), 5-iodo-2-methoxyphenol **22** (1 g, 4 mmol), triethylamine (0.51 g, 5 mmol), palladium acetate (9

mg, 0.04 mmol) and triphenylphosphine (21 mg, 0.08 mmol) were heated at 100°C. To the cooled reaction mixture was added aqueous hydrochloric acid (45 ml of a 2.7 M solution). After stirring for 10 min, the liquid was decanted off and the solid residue extracted with several portions of hot hexane. The combined hot hexane fractions were filtered. The cooled hexane solution was washed with water (2 x 10 ml), brine (10 ml), dried over magnesium sulfate, filtered and the solvent evaporated. Flash column chromatography (SiO_2 petrol : EtOAc 15:1) afforded the stilbene (**20**) as a white crystalline solid (109 mg, 16%). m.p. 99-100°C; $R_f = 0.34$ (SiO_2 petrol : EtOAc 1:1); δ_H (300 MHz) 2.28 (3 H, d, $J = 1.1$, CH_3), 3.89 (3 H, s, OCH_3), 3.90 (6 H, s, 2 x OCH_3), 3.94 (3 H, s, OCH_3), 5.61 (1 H, s, OH), 6.59 (2 H, s, H-2',6'), 6.74 (1 H, q, $J = 1.1$, olefinic H), 6.87 (1 H, d, $J = 8.7$, H-5''), 7.04 (1 H, dd, $J = 8.7$, 2.3, H-6''), 7.14 (1 H, d, $J = 2.3$, H-2''); Found C, 69.13; H, 6.71; $C_{19}H_{22}O_5$ requires C, 69.07; H, 6.71%; M^+, found 331.1541; $C_{19}H_{22}O_5$ (+H) requires 331.1545; λ_{max} (MeOH) = 296 (ε = 14,126).

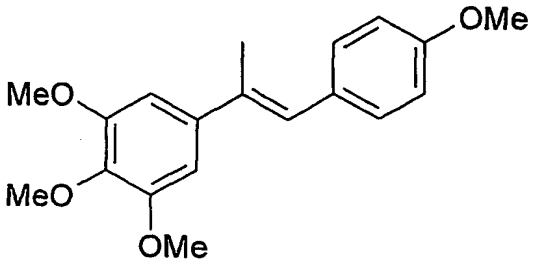
The ethyl derivative (**45**) has also been synthesised.



***Z*- and *E*-1-(4'-Methoxyphenyl)-2-(3'',4'',5''-trimethoxyphenyl)propene, 23a, 23b**



23a



23b

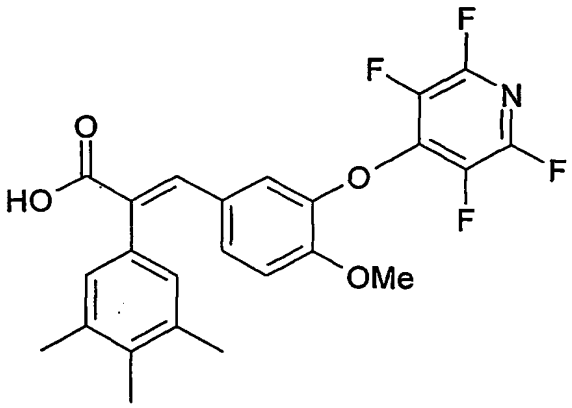
To a slurry of 4-methoxybenzylphosphonium chloride (598 mg, 1.43 mmol) in THF (8 ml) was added *n*-butyllithium (990 ml of 1.6 M solution, 1.58 mmol) at -15°C. The red anion was stirred for 20 minutes and 3,4,5-trimethoxyacetophenone (300 mg, 1.43 mmol) added. The resultant solution was stirred at room temperature for 1 hour and water (10 ml) carefully added. The aqueous layer was separated and extracted with ether (3 x 10 ml). The combined organic layers were washed with water (2 x 10 ml) and brine (10 ml), dried ($MgSO_4$) and concentrated *in vacuo*.

The nmr of the crude reaction product showed that the *Z*:*E* ratio was 1:1.5. Following flash column chromatography (SiO_2 petrol:EtOAc 9:1) the *Z* stilbene, **23a**, was isolated as white needles (45 mg, 0.143 mmol, 10%). m.p. 73-5°C; R_f = 0.46 (SiO_2 petrol:EtOAc 3:1); δ_H (300 MHz) 2.19 (1 H, d, *J* = 1.5, CH_3), 3.74 [6 H, s, $(OCH_3)_2$], 3.76 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 6.40 (1 H, q, *J* = 1.5, olefinic H), 6.42 (2 H, s, H-2',6'), 6.69 (1 H, dt, *J* = 8.7, 2.3, H-3'',5''), 6.93 (1 H, dt, *J* = 8.7, 2.3, H-2'',6''); λ_{max} (MeOH) = 273 (ε = 14,926).

Further elution afforded the *E* stilbene, **23b**, as an off white solid (44 mg, 0.140 mmol, 9.8%). m.p. 80-2°C; R_f = 0.41 (SiO_2 petrol:EtOAc 3:1); δ_H (300 MHz) 2.28 (1 H, d, *J* = 1.5, CH_3), 3.86 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 3.94 [6 H, s, $(OCH_3)_2$], 6.74 (2 H, s, H-2',6'), 6.75 (1 H, q, *J* = 1.5, olefinic H), 6.94 (1 H, dt, *J* = 8.7, 2.3, H-3'',5''), 7.34 (1 H, dt, *J* = 8.7, 2.3, H-2'',6''); λ_{max} (MeOH) = 287 (ε = 21,822).

Example 2: Synthesis of combretastatins with alkyl groups replacing the methoxy groups on the A ring

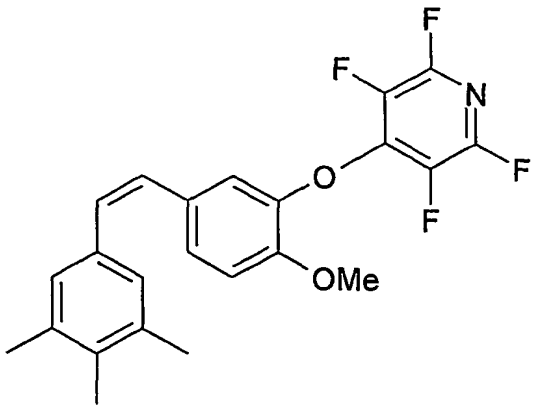
***E*-2-(3',4',5'-trimethylphenyl)-3-(3''-(2''',3''',5''',6'''-tetrafluoropyridoxy)-4''-methoxyphenyl)prop-2-enoic acid, 24**



24

A mixture of 3-(2',3',5',6'-tetrafluoropyridoxy)-4-benzaldehyde 25 (2 g, 6.64 mmol), 3,4,5-trimethylphenylacetic acid **26** (2.37 g, 13.3 mmol) acetic anhydride (6 ml) and triethylamine (3 ml) were heated under reflux for 3 h. After acidification with concentrated hydrochloric acid (9 ml), the solid was filtered off and recrystallised from ethanol to give *E*-2-(3',4',5-trimethyl)-3-(3''-(2''',3''',5''',6'''-tetrafluoropyridoxy)-4''-methoxyphenyl)prop-2-enoic acid **24** as a yellow crystalline solid (700 mg, 1.52 mmol, 23 %). m.p. 184-6°C. δ_H (300 MHz, DMSO) 2.11, (3 H, s, CH_3), 2.14 (6 H, s, $(CH_3)_2$), 3.83 (3 H, s, OCH_3), 6.61 (1 H, d, J = 1.5, H-2''), 6.71 (2 H, s, H-2',6'), 7.13 (1 H, d, J = 8.7, H-5''), 7.19 (1 H, dd, J = 8.7, 1.5, H-6''), 7.61, (1 H, s, olefinic H), 12.52, (1 H, s OH).

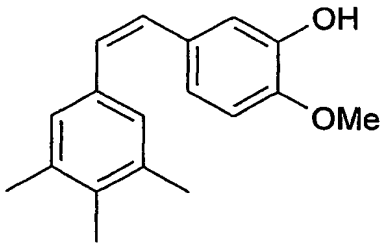
(*Z*)-1-(3',4',5'-trimethylphenyl)-2-(3''-(2''',3''',5''',6'''-tetrafluoropyridoxy)-4''-methoxyphenyl)ethene, 27



27

(*E*)-2-(3',4',5'-trimethylphenyl)-3-(3''-(2''',3''',5''',6'''-tetrafluoropyridoxy)-4''-methoxyphenyl)prop-2-enoic acid 24 (700 mg, 1.52 mmol) was added to powdered copper (500 mg, 7.81 mmol) in quinoline (5.5ml, 6.02 g, 28.2 mmol) and the resulting mixture was heated at 200 °C for 2 h. Upon cooling, ether was added and the copper filtered off through celite. The filtrate was washed with 1 M hydrochloric acid (2 x 20 ml) and the aqueous layer separated and extracted with ether (3 x 50 ml). The combined organic layers were washed with saturated sodium carbonate (50 ml), water (2 x 50 ml) and brine (50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Flash column chromatography (SiO_2 petrol:EtOAc 9:1) afforded (*Z*)-1-(3',4',5'-trimethylphenyl)-2-(3''-(2''',3''',5''',6'''-tetrafluoropyridoxy)-4''-methoxyphenyl)ethene **27** as a yellow oil (224 mg, 0.538 mmol, 35 %). $R_f = 0.48$ (SiO_2 petrol:EtOAc 9:1); δ_H (300 MHz) 2.15 (3 H, s, CH_3), 2.19 [6 H, s, $(CH_3)_2$], 3.84 (3 H, s, OCH_3), 6.41 (1 H, d, $J = 12.1$, olefinic H), 6.51 (1 H, d, $J = 12.1$, olefinic H), 6.87 (2 H, s, H-2',6'), 6.89 (1 H, d, $J = 8.7$, H-5''), 6.98 (1 H, d, $J = 2.3$, H-2''), 7.11 (1 H, dd, $J = 8.7, 2.3$, H-6'').

(*Z*)-1-(3',4',5'-trimethylphenyl)-2-(3''-hydroxy-4''-methoxyphenyl)ethene, 28



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To a solution of the (*Z*)-1-(3',4',5'-trimethylphenyl)-2-(3''-(2''',3''',5''',6'''-tetrafluoropyridoxy)-4''-methoxyphenyl)ethene (100 mg, 0.24 mmol) **27** in dry DMF (600 ml) and dichloromethane (115 ml) at 0°C was added sodium methoxide (25 mg, 0.463 mmol). After stirring overnight, the mixture was partitioned between ether (5 ml) and 1 M sulfuric acid (5 ml). The organic phase was washed with water (5 ml), dried ($MgSO_4$) and concentrated in vacuo. Flash column chromatography (SiO_2 petrol:EtOAc 9:1) and recrystallisation from petrol afforded (*Z*)-1-(3',4',5'-trimethylphenyl)-2-(3''-hydroxy-4''-methoxyphenyl)ethene as a white crystalline solid (31 mg, 0.116 mmol, 48 %). m.p. 60-1 °C. $R_f = 0.39$ (SiO_2 petrol:EtOAc 4:1);

δ_H (300 MHz) 2.16 (3 H, s, CH_3), 2.21 [6 H, s, $(CH_3)_2$], 3.90 (3 H, s, OCH_3), 5.50 (1 H, s, OH), 6.40 (1 H, d, J = 12.4, olefinic H), 6.45 (1 H, d, J = 12.4, olefinic H), 6.72 (1 H, d, J = 8.3, H-5''), 6.82 (1 H, dd, J = 8.3, 2.3, H-6''), 6.91 (1 H, d, J = 2.3, H-2''), 6.96 (2 H, s, H-2',6').

Example 3: Synthesis of combretastatins with a 3,4,5 trialkoxy group

***Z*- and *E*-1-(3',4',5'-triethoxyphenyl)-2-(3''-*t*-butyldimethylsilyloxy-4''-methoxyphenyl)ethene, 30a, 30b**

To a slurry of 3,4,5-triethoxybenzylphosphonium bromide **29** (2 g, 3.24 mmol) in THF (30 ml) was added n-butyllithium (2.5 ml of 1.6M solution in hexanes, 4 mmol) at –15°C under argon. The red anion was stirred for 20 min and 3-O-t butyldimethylsilyl-4-methoxybenzaldehyde **6** (0.86 g, 3.24 mmol) added. The resultant solution was stirred for 1 h at room temperature and water (10 ml) was carefully added. The aqueous layer was separated and extracted with ethyl acetate (3 x 100 ml). The combined organic layers were washed with water (2 x 100 ml), brine (100 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Flash column chromatography afforded the *cis* stilbene **30a** as a colourless oil (0.23 g, 15%). R_f = 0.65 (petrol:ethyl acetate 9:1); δ_H (300 MHz) 0.08 (6 H, s, $Si(\underline{CH_3})_2$), 0.95 (9 H, s, 3 x CH_3), 1.35 (9 H, m, 3 x $OCH_2\underline{CH_3}$), 3.79 (3 H, s, OCH_3), 3.91 (4 H, q, J = 6.8, CH_2), 4.06 (2 H, q, J = 7.2, CH_2), 6.40 (1 H, d, J = 12.1, olefinic H), 6.43 (1 H, d, J = 12.1, olefinic H), 6.74 (2 H, s, ArH 2, 6), 6.83 (1 H, dd, J = 8.0, 2.1, ArH *para* to OSi), 6.84 (1 H, d, J = 8.0, ArH *ortho* to OMe), 6.88 (1 H, d, J = 2.1, ArH *ortho* to OSi).

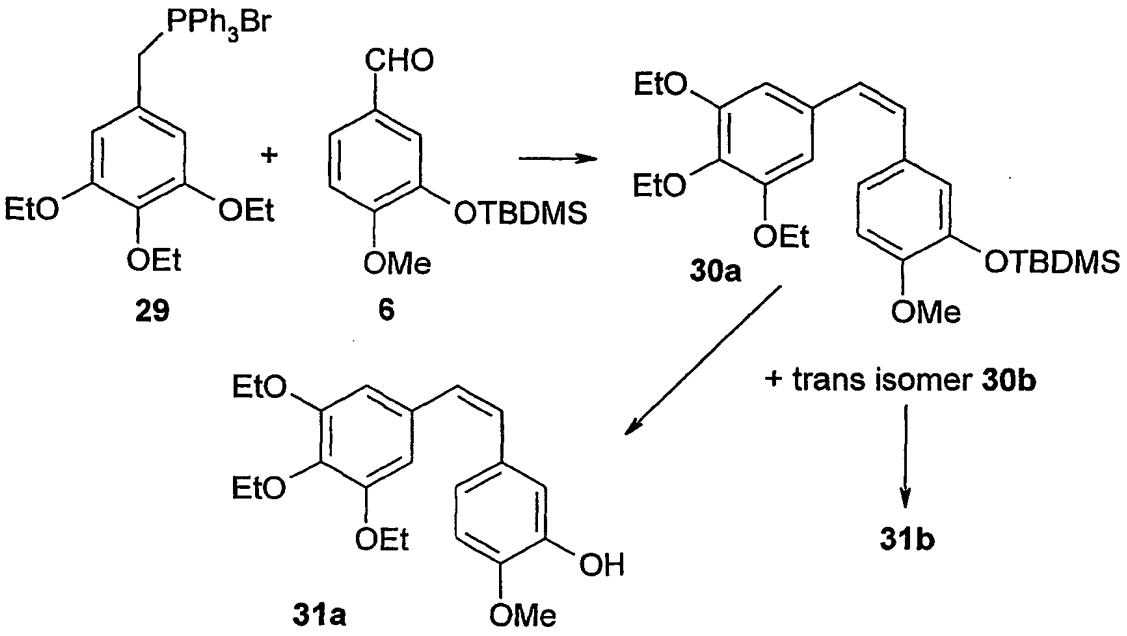
Further elution gave the *trans* stilbene **30b** as white crystals (0.27 g, 17.6%). R_f = 0.75; δ_H (300 MHz) 0.20 (6 H, s, $Si(\underline{CH_3})_2$), 1.03 (9 H, s, 3 x CH_3), 1.37 (3 H, t, J = 7.5, $CH_2\underline{CH_3}$), 1.46 (6 H, t, J = 7.2, 2 x $CH_2\underline{CH_3}$), 3.85 (3 H, s, OCH_3), 4.12 (6 H, m, 3 x CH_2), 6.73 (2 H, s, ArH 2, 6), 6.84 (1 H, dd, J = 7.8, 1.98, ArH *para* to OSi), 6.90 (1 H, d, J = 15.1, olefinic H), 7.04 (1 H, d, J = 7.8, ArH *ortho* to OMe), 7.05 (1 H, d, J = 15.1, olefinic H), 7.66 (1H, d, J = 2.0, ArH ortho to OSi).

***Z*- and *E*-1-(3',4',5'-triethoxyphenyl)-2-(3''-hydroxy-4''-methoxyphenyl)ethene, 31a, 31b**

To a stirred mixture of *cis* and *trans*-1-(3',4',5'-triethoxyphenyl)-2-(3''-*tert*-butyldimethylsiloxy-4''-methoxy)ethene **30a, 30b** (0.23 g, 0.48 mmol - *cis* isomer; 0.27 g, 0.57 mmol - *trans* isomer) in dry THF (17.5 ml) was added tetra-n-

butylammonium fluoride (1.46 ml of 1 M solution in THF). The resulting yellow solution was stirred for twenty min and treated with water (50 ml). The aqueous layer was separated and extracted with chloroform (3 x 50 ml). The combined organic layers were washed with water (2 x 50 ml) and brine (50ml), dried ($MgSO_4$) and concentrated *in vacuo*.

Flash column chromatography (petrol:ethyl acetate 4:1) afforded *Z*- 1-(3',4',5'-triethoxyphenyl)-2-(3''-hydroxy-4''-methoxyphenyl)ethene **31a** as a colourless oil (0.08 g, 46%). R_f = 0.24. δ_H (300 MHz) 1.34 (9 H, m, 3 x $CH_2\underline{CH_3}$), 3.87 (3 H, s, OCH_3), 3.92 (4 H, q, *J* = 7.2, 2 x CH_2), 4.06 (2 H, q, *J* = 7.2, CH_2), 6.40 (1 H, d, *J* = 12.43, olefinic H), 6.45 (1 H, d, *J* = 12.4, olefinic H), 6.50 (2 H, s, ArH 2,6), 6.75 (1 H, d, *J* = 8.1, ArH *ortho* to OMe), 6.79 (1 H, dd, *J* = 8.7, 1.88, ArH *para* to OH), 6.91 (1 H, d, *J* = 1.9, ArH *ortho* to OH). M^+, 358.



Further elution afforded *E* - 1-(3',4',5'-triethoxyphenyl)-2-(3''-hydroxy-4''-methoxyphenyl)ethene **31b** (0.09 g, 46.6%) as white crystals. mp: 91-92°C; Rf = 0.11. δ_H (300 MHz) 1.37(3 H, t, *J* = 6.0, CH_3), 1.46 (6 H, t, *J* = 6.4, 2 x CH_3), 3.92 (3 H, s, OCH_3), 4.12 (6 H, m, 3 x CH_2), 6.71 (2 H, s, H 2, 6), 6.84 (1 H, d, *J* = 7.8, ArH *ortho* to OMe), 6.85 (1 H, d, *J* = 15.5, olefinic H), 6.91 (1 H, dd, *J* = 7.9, 2.26, ArH *para* to OH), 7.11 (1 H, d, *J* = 15.5, olefinic H), 7.13 (1 H, d, *J* = 2.3, ArH *ortho* to OH). M^+, 358.

***Z*- and *E*-1-(3',4',5'-triethoxyphenyl)-2-(3''-fluoro-4''-methoxyphenyl)ethene, 32a, 32b**

To a slurry of 3,4,5-triethoxybenzylphosphonium bromide **29** (2 g, 3.24 mmol) in THF (30 ml) was added n-butyllithium (2.5 ml of 1.6 M solution in hexanes, 4 mmol) at -15°C under argon. The red anion was stirred for 20 min and 3-fluoro-4-methoxybenzaldehyde (0.50 g, 3.24mmol) was added. The resultant solution was stirred for 1 h and water (10 ml) was carefully added. The aqueous layer was separated and extracted with ethyl acetate (3 x 100 ml). The combined organic layers were washed with water (2 x 100 ml) and brine (100 ml), dried ($MgSO_4$) and concentrated *in vacuo*.

Flash column chromatography (SiO_2, petrol:ethyl acetate 20:1) afforded the *cis* –stilbene **32a** as a pale yellow oil (0.35 g, 29%), R_f = 0.16; δ_H (300 MHz) 1.35 (9 H, m, 3 x CH_3), 3.87 (3 H, s, OCH_3), 3.92 (4 H, q, J = 6.8, 2 x CH_2), 4.07 (2 H, q, J = 7.2, CH_2), 6.41 (1 H, d, J = 12.4, olefinic H), 6.47 (1 H, d, J = 12.4, olefinic H), 6.47 (2 H, s, ArH 2,6), 6.84 (1 H, t, J = 8.7, ArH *ortho* to OMe), 7.00 (1 H, dd, J = 8.7, 1.5, ArH *para* to F), 7.05 (1 H, d, J = 12.1, 1.5, ArH *ortho* to F). M^+, 360.

Further elution afforded the *trans* isomer **32b** (0.27 g, 23 %) as white needles mp:97-99°C; Rf = 0.22; δ_H (300 MHz) 1.38 (6 H, t, J = 7.2, 2 x CH_3), 1.46 (3H, t, J = 7.2, CH_3) 3.92 (3 H, s, OCH_3), 4.12 (6 H, m, 3 x CH_2), 6.71 (2 H, s, H 2,6) 6.91 (1 H, d, J = 15.5, olefinic H), 6.94 (1 H, t, J = 8.7, ArH ortho to OMe), 6.95 (1 H, d, J = 15.5, olefinic H), 7.17 (1 H, dd, J = 8.7, 2.3 ArH *para* to F), 7.25 (1 H, dd, J = 12.0, 2.3, ArH *ortho* to F). M^+, 360.

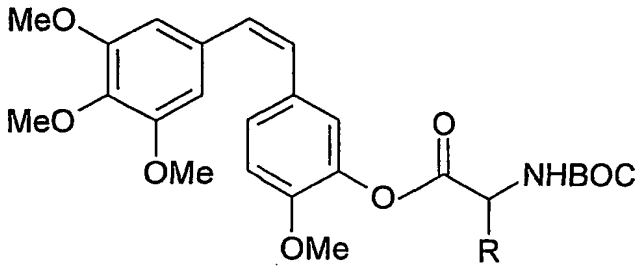
Example 4: Boc-combretastatin compounds

Boc-Phenylalanine combretastatin A-4, 33

To a stirred solution of *t*-butoxycarbonyl-phenylalanine (168 mg, 0.634 mmol), dicyclohexylcarbodiimide (157 mg, 0.76 mmol), N,N- 4-dimethylaminopyridine (8 mg, 61 μmol) in dichloromethane (15 ml) under nitrogen at room temperature in the dark was added combretastatin A-4 (**1**) (200 mg, 0.633 mmol). After stirring for 48 h, the mixture was filtered, evaporated and the residue chromatographed on silica using

petroleum (bp 40-60°C)/ethyl acetate 4:1 to afford the title ester **(33)** as a clear gum (143 mg, 40%). ν_{max} 3364 (NH); 1766, 1716 (C=O). δ_H 7.22 (5 H, m, phenylalanine ArHs); 7.11 (1H, dd, J = 8.1, 2.0, H *para* to O ester); 6.95 (1 H, d, J = 2.0, H *ortho* to O ester); 6.83 (1 H, d, J = 2.0, H *meta* to O ester); 6.40, 6.50 (4 H, 2 s, olefinic Hs, A-ring Hs); 5.07 (1 H, broad, NH); 4.80 (1 H, m, H-α); 3.80, 3.70, 3.66 (12 H, 3 x s, 4 x OMe);3.35 - 3.08 (2 H, m, CH_2); 1.40 (9 H, s, 3 x CH_3). λ_{max} 241; 271; 305. *m/z* 463 (35%, M + H - BOC); 317 (100).

The other BOC compounds **34** - **44** were made using the above method.



BOC = *t*-butoxycarbonyl.

33	R = Phe	39	R = Ala
34	R = Ile	40	R = His
35	R = Gly	41	R = Pro
36	R = Trp	42	R = D-Met
37	R = Met	43	R = D-Trp
38	R = Leu	44	R = Tyr

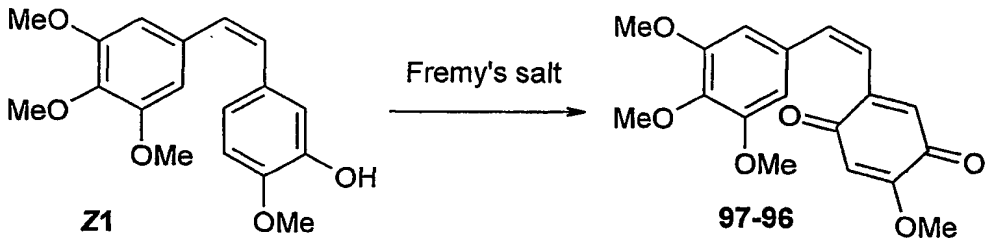
Example 5: Benzoquinone compounds

2-Methoxy-5-[(*Z*)-2-(3',4',5'-trimethoxyphenyl)-vinyl]-[1,4]benzoquinone 97-96

To a mixture of Aliquat 336 (0.181 ml, 1.25 equiv) and $NaH_2PO_4.H_2O$ (323 mg, 2.34 mmol) in water (100 ml) was added a solution of combretastatin A4 (**1**) (100 mg, 0.316 mmol) in dichloromethane (7 ml). Fremy's salt (potassium nitrosodisulfonate) (212 mg, 0.8 mmol) was added and the mixture shaken for 30 min. (Colour changes from mauve to red). The dichloromethane was collected and the aqueous fraction extracted with dichloromethane (3 x 10 ml). The combined organic phases were

washed with water (3 x 10 ml), brine (10 ml) and dried over magnesium sulfate. Evaporation of the solvent followed by flash chromatography of the residue using petrol:ethyl acetate (65:35) as eluent afforded the quinone as a red crystalline solid (51 mg, 49%) mp 130-2°C, δ_H (acetone d6) 3.70, 3.75, 3.85 (12 H, 3 s, 4 x OMe); 6.08 (1 H, s, quinone-H *ortho* to OMe); 6.43 (1 H, dd, *J* 12.5, 0.5, olefinic-H next to quinone ring); 6.67 (1 H, d, *J* 0.5, quinone-H *meta* to OMe); 6.73 (2 H, s, ArHs); 6.96 (1 H, d, *J*, 12.5, olefinic-H next to Ar ring). λ_{max} 296 (ε12,708); 470 (2038). ν_{max} 1666, 1647 cm^{-1}. M+, 330 (40%); 315 (M – Me, 60); 69 (100).

This method was also used to synthesise 98-40, 98-23, 98-33 and 98-24.



These quinones may act as prodrugs producing the active hydroquinones on reduction by enzymes.

Example 6: Biological Activity of Compounds

The following assays (1-5) were carried out as described in our paper (Woods, et al, British Journal of Cancer, 71, 705-711 (1995)). In addition to the cell lines described in this paper, other established human cell lines (K562, HUVEC, H460, BE, H529, and HT29) were used in the cytotoxicity/growth inhibition assay.

(1) Inhibition of tubulin assembly. The figure quoted is the concentration required to reduce assembly of tubulin by 50%. Tubulin assembly is monitored by light scatter/absorption at 350 nm.

(2) Competition for the colchicine binding site on tubulin. The figure quoted represents the % of ^{3}H-colchicine bound following co-incubation of test compound

and ^{3}H-colchicine with isolated tubulin. Where ester pro-drugs were used the experiments were carried out in the presence and absence of porcine liver esterase.

(3) Cytotoxicity/growth inhibition assay. This was carried out by the MTT assay.

(4) Permeability (shape-change) assay in endothelial cells. This was carried out using a method based on that of Watts et al. (Anticancer Res., 17, 71-75, (1997)). This involved the diffusion of a fluorescently labelled dextran through a barrier of endothelial cells (HUVEC) grown to confluence on a porous membrane. The effect of agents to alter the shape of these cells results in an increase in the permeability of the cells to the dye. The figure represents the increase in permeability over control cultures when drug is added (30 minutes, 1mM.).

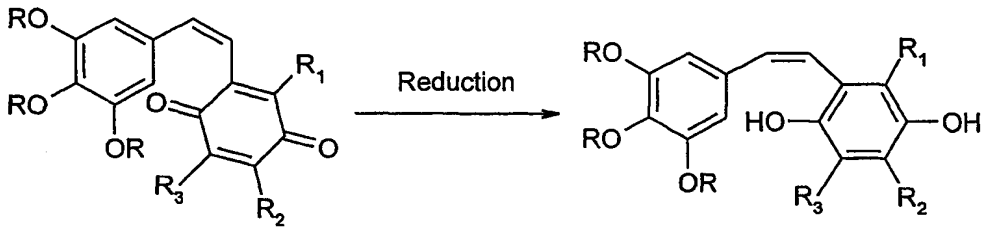
(5) Experiments to study the anti-vascular effects of these agents were carried out as described previously by our group. (Zhao et al, European Journal of Nuclear Medicine, 26, 231-238 (1999)). The anti-vascular effects of the agents were monitored by either positron emission tomography (PET) or by histologogical examination following treatment of mice bearing T50:80 murine breast tumours, or H460 human lung tumours.

(6) Anti-tumour efficacy of agents was determined in xenografts of H460 human lung cancer. Animals (n=5) were treated either with 5 daily injections and tumour size measured with time following treatment.

(7) The pharmacokinetic profile of agents was determined in mice following injection with drug. Blood was taken at various times following treatment and analysed by HPLC with UV detection. The HPLC conditions were isocratic using a 5 micron C18 BDS column (150 X 4.6) and eluting with 75%MeOH in water with a detection wavelength of 290 nm. The retention time of 6.6 mins for 97-64H.

(8) Activation of quinone prodrugs by DT-diaphorase. (NAD(P)H:quinone

oxidoreductase, EC 1.6.99.2). DT-diaphorase is an enzyme over-expressed in a number of human tumours. We have recently shown this enzyme to be highly expressed in the endothelial cells of blood vessels. This two-electron reducing enzyme can be used to selectively activate prodrugs within cancers which over-express the enzyme. We have therefore synthesised quinone pro-drugs (97-96, 98-40, 98-23, 98-33, 98-24) which, upon reduction by the enzyme, produces an agent (a hydroquinone stilbene) which is cytotoxic.



Quinone Hydroquinone

Results

The *in vitro* studies reported in Table 11 illustrate the structural requirements necessary for biological activity. Modifications of the A ring have shown that the methoxy groups can be replaced by alkoxy or alkyl (28,31a,32a) whilst retaining their ability to inhibit assembly of isolated tubulin. Similarly, molecules with alkyl substituents on the ethene bridge (19,20,46) retain activity both as inhibitors of tubulin assembly and are growth inhibitory *in vitro*. The pharmacophore consists of a stilbene in a *cis* configuration with a small alkyl or O-alkyl substituent at the 4-position of the B ring. Substitution at the 3-position of the B-ring with F results in highly active compounds that are potent inhibitors of tubulin assembly, and are potently growth inhibitory (97-64H, and 98-35). These agents show good activity in the shape-change (permeability) assay. This test is used as an *in vitro* assay of vascular damage. 97-64H also shows anti-tumour activity *in vivo* in H460 human lung cancer xenografts. 97-64H was given at either 2/3 of the maximum tolerated dose (MTD =200mg/kg) or at ¾ of the MTD to mice bearing liver metastatic T50:80 tumours. Tumours were removed and examined for evidence of vascular damage at 2hr and 4 hr following drug administration. Similarly a dose equivalent to ¾ of the MTD was

administered and the tumours examined 24hr and 48hr following administration of drug. Examination of these tumours showed tumour necrosis, blocked vessels, infiltration with red blood cells, consistent with damage to the vasculature. These effects are seen within 2 hours. 97-64H is orally bio-available when administered at 200mg/kg in 5% dimethylacetamide in arachis oil, 97-64H showed a Cmax of 1.49 ug/ml, an absorption half life 15 mins, and an elimination half life 32 mins. The concentration achieved *in vivo* (1.49 mg/ml = 4.6 mM) is far in excess of the concentration necessary to inhibit the growth of HUVEC cells *in vitro* (0.001mM) indicating that this agent is bio-available when administered orally.

The 3-position of the B-ring can also be substituted with larger groups such as boc-amino acid esters, pyridyl esters, and ethers. The amino acid esters (33-44) are prodrugs which, upon the action of esterase, release the active agent. The activity of these agents is related to the rate of hydrolysis of these agents by esterase. The most active compounds being those which are most readily hydrolysed. The activity of these compounds indicates that ester linked polymers and peptides would be good prodrugs for agents of this type (see Table 1).

The pyridyl esters (96-167 and 97-07) are potent inhibitors of tubulin assembly and can displace ^{3}H-colchicine from tubulin without the action of esterase, showing that the 3-position of the B-ring can be substituted with bulky side groups. Potent growth inhibitory activity and good activity in the shape-change assay can also be seen for the tetrafluoropyridyl ether (97-13) and other ethers (98-29) which are not a substrate for esterase.

A series of prodrugs capable of being activated by DT-diaphorase, an enzyme over-expressed in a wide range of human cancers and in endothelial cells of the vasculature were synthesised. The rationale for this is that these agents will be activated in situ by the tumours over-expressing the enzyme, thus giving rise to a high local concentration of active drug. This confers selectivity to this agent. These quinone prodrugs (97-96 and 98-23) were tested for their ability to act as substrates for DT-diaphorase. It can

be seen that these compounds are good substrates for the enzyme, and are comparable to RH1 (2,5,-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone) a novel alkylating agent which is activated by DT-diaphorase and is currently about to enter clinical trial. An analysis of the growth inhibitory properties of these agents shows that 97-96 is 16-fold more active in the H460 human lung cell line which expresses active DT-diaphorase than in the diaphorase null H596 cell line 97-96 is active in an H460 human lung tumour xenograft, a tumour which expresses active DT-diaphorase. This agent may therefore have a role in the treatment of tumours that over-express DT-diaphorase. Similarly, these agents maybe selectively activated in the endothelial cells of the vasculature, thereby conferring a selective anti-vascular effect.

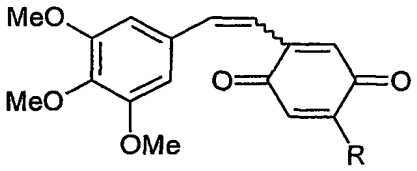
Table 1:

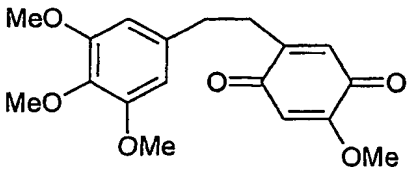
Compound	IC_{50} (nM) A2780	IC_{50} (nM) A2780/ADR	Inhibition of tubulin assembly (IC_{50} μM)	Colchicine displacement protein:drug 1:10 (with esterase)	Colchicine displacement protein:drug 1:10(without esterase)
1	0.72	0.84	2.4	NA	12
33	2.8	4.0	3.6*	2	92
34	>60	>60	6.2*	2	65
35	4.3	5.0	7.5*	4	65
36	1.7	2.0	4.0*	ND	ND
37	6.7	9.1	6.0*	2	88
38	14.0	27.0	7.8*	ND	72
39	2.7	4.6	8.0	2	77
40	2.2	4.3	7.0*	4	2
41	29.0	39.0	9.0*	2	78
42	2.9	2.9	3.5*	2	ND
43	3.3	3.8	3.0*	2	ND
44	4.5	6.4	ND	ND	ND

Table 2:

Compound	P388	A2780	H460	H596	H596/H460
A-4	2.6	0.72	1.51		
97-96	570	190	38	620	16
98-40	>5000	4630	>5000	2190	<0.44
98-23	2180	2150	4370	2280	0.52
98-33	<780	950	4110	1490	0.36
98-24	17050	1630	3080	1950	0.63

IC_{50} are in nM.





97-96 R = OMe (cis)

98-40 R = OMe (trans)

98-23 R = Me (cis)

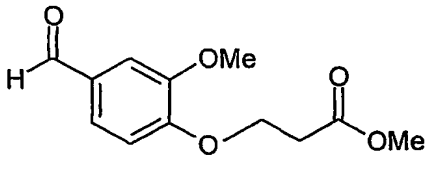
98-33 R = Me (trans)

98-24

Example 7: Synthesis of combrestatatin A-4 bearing a photocleavable group

Methyl 4-(4'-formyl-2'-methoxyphenoxy) butanoate 4

(see D. L. McMinn and M. M. Greenberg, Tetrahedron, 1996, 52, 3827)



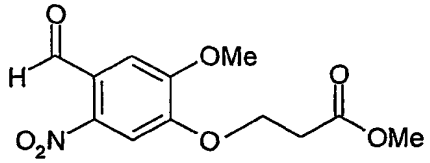
4

To a stirred solution of vanillin (**3**)(2 g, 13.16 mmol) and methyl 4-bromobutyrate (2.38 g, 13.16 mmol) in DMF (20 cm^3) was added freshly ground anhydrous

potassium carbonate (2.03 g, 14.47 mmol). The resulting pale pink suspension was heated at 100°C for 90 mins, after which time the suspension has become milky pink in appearance. The mixture was then poured into water (50 cm^3) and extracted with Et_2O (3 x 30 cm^3). The combined organic extracts were washed with water (2 x 30 cm^3), then 1M HCl (2 x 30 cm^3) before being dried ($MgSO_4$) and concentrated *in vacuo*. The pale pink solid **4** was used without further purification (2.7 g, 82 %). m.p. 69°C [lit. (D. L. McMinn and M. M. Greenberg, *Tetrahedron*, 1996, **52**, 3827) m.p. 68-69°C]; Found C, 62.0; H, 6.3; $C_{13}H_{16}O_5$ requires C, 62.0; H, 6.4%; R_f 0.44 (SiO_2, hexane:EtOAc, 2:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3100-2700 (m), 1740 (s), 1680 (s); δ_H (300 MHz, $CDCl_3$) 2.19 (2H, q, *J* 6.6 Hz, CH_2), 2.56 (2H, t, *J* 6.6 Hz, CH_2), 3.68 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.15 (2H, t, *J* 6.6 Hz, CH_2) , 6.97 (1H, d, *J* 8.0 Hz, H-6'), 7.40 (1H, dd, *J* 8.0 Hz, *J* 1.8 Hz, H-5'), 7.44 (1H, d, *J* 1.8 Hz, H-3'), 9.84 (1H, s, CHO) ppm; δ_C (100 MHz, $CDCl_3$) 24.1 (CH_2), 30.2 (CH_2), 51.5 (OCH_3), 55.8 (OCH_3), 67.3 (CH_2), 109.2 (CH), 111.5 (CH), 126.6 (CH), 130.0, 149.7, 153.7, 173.3 ($\underline{C}OOCH_3$), 190.8 (CHO) ppm; *m/z* (FAB) 253 [(MH^+), 50%].

Methyl 4-(4'-formyl-2'-methoxy-5'-nitrophenoxy) butanoate 5

(see D. L. McMinn and M. M. Greenberg, *Tetrahedron*, 1996, 52, 3827.)



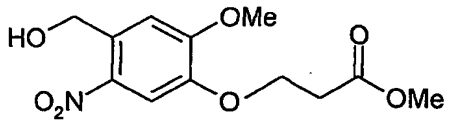
5

To a pale pink solution of methyl 4-(4'-formyl-2'-methoxyphenoxy) butanoate (**4**) (3 g, 12.3 mmol) in DCM (20 cm^3) was added dropwise, at 0°C fuming nitric acid (1.5 cm^3, 37.0 mmol). The resulting green solution was stirred at 0°C for a further 30 mins before being allowed to spontaneously warm to r.t., where it remained for 3 hours. The subsequent bright yellow suspension was poured onto iced water (50 cm^3) and extracted with DCM (3 x 30 cm^3). The combined organic phase was washed with sat. $NaHCO_3$ solution (2 x 50 cm^3), followed by water (2 x 50 cm^3) before being dried and concentrated *in vacuo* providing a bright yellow solid. The desired compound **5** was used without further purification (2.48 g, 68 %). A small amount of material (250 mg,

0.84 mmol) was purified *via* column chromatography (SiO_2, CH_2Cl_2) so that complete characterisation data could be obtained. m.p. 75°C [lit. (D. L. McMinn and M. M. Greenberg, *Tetrahedron*, 1996, 52, 3827.) m.p. 76-78°C]; Found C, 52.7; H, 5.2; N, 4.8; $C_{13}H_{15}NO_7$ requires C, 52.5; H, 5.1; N, 4.7%; R_f 0.44 (SiO_2, CH_2Cl_2); ν_{max} (KBr disc)/cm^{-1} 3100-2700 (m), 1735 (s), 1690 (s), 1290, 1220; δ_H (200 MHz, $CDCl_3$) 2.23 (2H, q, *J* 7.1 Hz, CH_2), 2.57 (2H, t, *J* 7.1 Hz, CH_2), 3.71 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.21 (2H, t, *J* 7.1 Hz, CH_2), 7.40 (1H, s, H-3'), 7.61 (1H, s, H-6'), 10.44 (1H, s, CHO) ppm; δ_C (100 MHz, $CDCl_3$) 24.1 (CH_2), 30.2 (CH_2), 51.8 (OCH_3), 56.6 (OCH_3), 59.6 (CH_2), 108.1 (CH), 109.9 (CH), 125.5, 143.8, 151.7, 153.4, 173.2 ($\underline{C}OOCH_3$), 187.8 (CHO) ppm; *m/z* (FAB) 298 [(MH^+), 40%].

Methyl 4-[4'-(hydroxymethyl)-2'-methoxy-5'-nitrophenoxy] butanoate 6

(see D. L. McMinn and M. M. Greenberg, *Tetrahedron*, 1996, 52, 3827.)

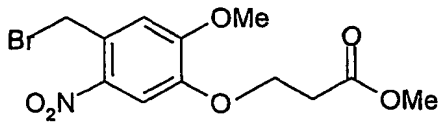


6

To a clear yellow solution of methyl 4-(4'-formyl-2'-methoxy-5'-nitrophenoxy) butanoate (**5**)(1 g, 3.36 mmol) in THF (10 cm^3) was added, portion wise, sodium borohydride (128 mg, 3.36 mmol). The solution quickly changed appearance, becoming deep orange after about 5 mins. The mixture was stirred for a further 55 mins before water was added (25 cm^3), the subsequent yellow mixture was extracted with ether (3 x 30 cm^3) and the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* providing a pale yellow solid. The solid, **6** was used without further purification (875 mg, 87.5 %). A small amount of material (200 mg, 0.67 mmol) was purified *via* column chromatography (SiO_2, hexane:EtOAc 2:1 v/v) so that complete characterisation data could be obtained. m.p. 100°C [lit. (D. L. McMinn and M. M. Greenberg, *Tetrahedron*, 1996, 52, 3827.) m.p. 98-100°C]; Found C, 52.2; H, 5.7; N, 4.7; $C_{13}H_{17}NO_7$ requires C, 52.2; H, 5.7; N, 4.7%; R_f 0.24 (SiO_2, hexane:EtOAc 2:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3400-3100 (br), 3000-2800 (m), 1730 (s), 1280, 1220; δ_H (200 MHz, $CDCl_3$) 2.20 (2H, q, *J* 7.3 Hz, CH_2), 2.56 (2H, t, *J* 7.3

Hz, CH_2), 2.61 (1H, br, OH), 3.70 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.13 (2H, t, *J* 7.3 Hz, CH_2) , 4.95 (2H, s, CH_2), 7.15 (1H, s, H-3'), 7.71 (1H, s, H-6') ppm; δ_C (100 MHz, $CDCl_3$) 24.3 (CH_2), 30.4 (CH_2), 51.7 (OCH_3), 56.4 (OCH_3), 62.8 (CH_2), 68.3 (CH_2), 109.5 (CH), 111.2 (CH), 132.4, 139.6, 147.2, 154.3, 173.4 ($\underline{C}OOCH_3$) ppm; *m/z* (FAB) 299 [(M^+), 15%], 282 [(M-OH), 40%].

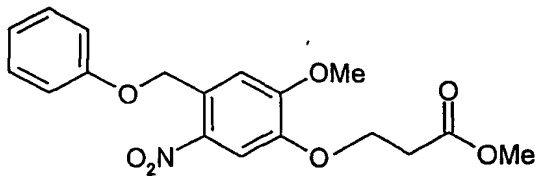
Methyl 4-[4'-(bromomethyl)-2'-methoxy-5'-nitrophenoxy] butanoate 7



7

To a solution of methyl 4-[4'-(hydroxymethyl)-2'-methoxy-5'-nitrophenoxy] butanoate (**6**) (750 mg, 2.52 mmol) in anhydrous THF (10 cm^3) was added PBr_3 (0.24 cm^3, 2.52 mmol). The resulting deep yellow solution was refluxed for 3 hours, after which time no visible changes had occurred. The reaction mixture was cooled then poured onto ice. The resulting aqueous solution was extracted with ether (3 x 30 cm^3). The combined organic extracts were washed with 5% $NaHCO_3$ solution (2 x 30 cm^3) and water (2 x 30 cm^3) before being dried ($MgSO_4$) and concentrated *in vacuo*. The desired compound 7 was furnished as a yellow oil. The oil was then purified *via* column chromatography (SiO_2, hexane:EtOAc, 2:1 v/v) thus providing 7 as a yellow powder (580 mg, 64 %). m.p. 68-70°C; Found C, 43.2; H, 4.5; N, 4.0; Br, 21.9; $C_{13}H_{16}NO_6Br$ requires C, 43.1; H, 4.4; N, 3.9; Br, 22.1%; R_f 0.63 (SiO_2, hexane:EtOAc 2:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3000-2800 (m), 1720 (s), 1610, 1580, 1530, 1280, 1220; δ_H (200 MHz, $CDCl_3$) 2.20 (2H, q, *J* 6.7 Hz, CH_2), 2.56 (2H, t, *J* 6.7 Hz, CH_2), 3.70 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.13 (2H, t, *J* 6.7 Hz, CH_2) , 4.86 (2H, s, CH_2), 6.92 (1H, s, H-3'), 7.67 (1H, s, H-6') ppm; δ_C (100 MHz, $CDCl_3$) 24.1 (CH_2), 30.1 (CH_2), 51.6 (OCH_3), 56.4 (OCH_3), 68.0 (CH_2), 68.3 (CH_2), 109.7 (CH), 113.8 (CH), 127.3, 140.0, 148.1, 153.5, 173.2 ($\underline{C}OOCH_3$) ppm; *m/z* (FAB) 362 [(MH^+), 100%].

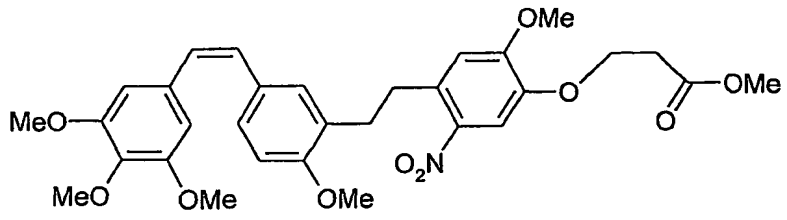
Methyl 4-[2'-methoxy-5'-nitro-4'-(phenoxymethyl)phenoxy] butanoate 9



9

To a suspension of phenol (130 mg, 138 mmol) and methyl 4-[4'-(bromomethyl)-2'-methoxy-5'-nitrophenoxy] butanoate (7) (500 mg, 138 mmol) in anhydrous methanol (5 cm^3) was added K'OBu (186 mg, 166 mmol). The resulting green mixture was stirred at r.t. for 30 mins after which time a white precipitate had formed. The precipitate was filtered and recrystallised from methanol affording the desired compound **9** as a pure white solid (319 mg, 61%). m.p. 96-98°C; Found C, 61.0; H, 5.8; N, 4.0; $C_{19}H_{21}NO_7$ requires C, 60.8; H, 5.6; N, 3.7%; R_f 0.64 (SiO_2, hexane:EtOAc 2:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3000-2800 (m), 1720 (s), 1610, 1580, 1520, 1280, 1220; δ_H (300 MHz, $CDCl_3$) 2.21 (2H, q, *J* 6.7 Hz, CH_2), 2.57 (2H, t, *J* 6.7 Hz, CH_2), 3.71 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.14 (2H, t, *J* 6.7 Hz, CH_2) , 5.50 (2H, s, CH_2), 6.98-7.02 (3H, m, ar), 7.29-7.34 (3H, m, ar), 7.77 (1H, s, ar) ppm; δ_C (100 MHz, $CDCl_3$) 24.7 (CH_2), 30.4 (CH_2), 51.7 (OCH_3), 56.3 (OCH_3), 67.0 (CH_2), 68.2 (CH_2), 109.4 (CH), 109.6 (CH), 115.0 (CH), 121.5 (CH), 129.6 (CH), 129.7 (CH), 138.9, 147.0, 154.3, 158.1, 173.2 ($COOCH_3$) ppm; *m/z* (FAB) 376 [(MH^+), 20%], 282 [($M-C_6H_5O$), 80%].

Methyl 4-[2'-methoxy-4'-({2''-methoxy-5''-[(*Z*)-2'''-3''',4''',5'''-trimethoxyphenyl)ethenyl]phenoxy}-methyl)-5'-nitrophenoxy]butanoate *Z*-8

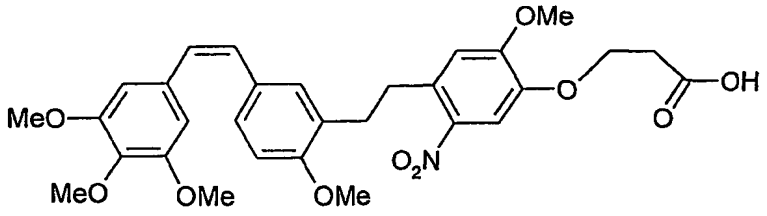


***Z*-8**

To a suspension of methyl 4-[4'-(bromomethyl)-2'-methoxy-5'-nitrophenoxy] butanoate (7) (1.5 g, 4.14 mmol) and *cis*-CA-4 (1.6 g, 5.0 mmol) in anhydrous

methanol (20 cm^3) was added K'OBu (0.58 g, 5.2 mmol). The resulting yellow mixture was stirred at r.t.. for 30 mins after which time a yellow precipitate had formed. The precipitate was filtered and recrystallised from methanol furnishing the desired compound ***Z*-8** as a pale yellow solid (1.62 g, 69%). m.p. 132-134°C; Found C, 65.2; H, 6.5; N, 2.4; $C_{31}H_{35}NO_{11}$ requires C, 65.6; H, 6.2; N, 2.5%; Accurate mass; found M^+ 597.2201; $C_{31}H_{35}NO_{11}$ requires M^+ 597.2210; R_f 0.60 (SiO_2, hexane:EtOAc 2:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3000-2800 (m), 1730 (s), 1610, 1580, 1520, 1320, 1280, 1220, 1130, 880; λ_{max}(MeCN)/nm 222 (ε 35050), 242 (ε 29263) and 293 (ε 14071); δ_H (300 MHz, $CDCl_3$) 2.21 (2H, q, *J* 6.7 Hz, CH_2), 2.58 (2H, t, *J* 6.7 Hz, CH_2), 3.69 (6H, s, 2 x OCH_3), 3.71 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.51 (2H, t, *J* 6.7 Hz, CH_2), 5.41 (2H, s, CH_2), 6.45 (1H, d, *J* 12.4 Hz, olefinic H), 6.49 (1H, d, *J* 12.4 Hz, olefinic H), 6.50 (2H, s, 2'''-H and 6'''-H), 6.82 (1H, d, *J* 8.3 Hz, H-3''), 6.93-6.97 (2H, m, H-4'' and H-6''), 7.46 (1H, s, H-3'), 7.75 (1H, s, H-6') ppm; δ_C (100 MHz, $CDCl_3$) 24.3 (CH_2), 30.4 (CH_2), 51.7 (OCH_3), 55.9 (OCH_3), 56.0 (OCH_3), 56.2 (OCH_3), 60.9 (OCH_3), 68.2 (CH_2), 68.4 (CH_2), 105.9 (CH), 109.3 (CH), 109.7 (CH), 111.6 (CH), 115.6 (CH), 123.0 (CH), 129.2 (CH), 129.4 (CH), 129.6, 130.3, 132.6, 137.2, 138.8, 147.0, 147.3, 149.0, 152.9, 154.3, 173.4 ($\underline{C}OOCH_3$) ppm; *m/z* (FAB) 376 [($M^{+\cdot}$), 40%].

4-[2'-methoxy-4'-({2''-methoxy-5''-[(Z)-2'''-3''',4''',5'''-trimethoxyphenyl)ethenyl]phenoxy}methyl)-5'-nitrophenoxy]butanoic acid *Z*-11

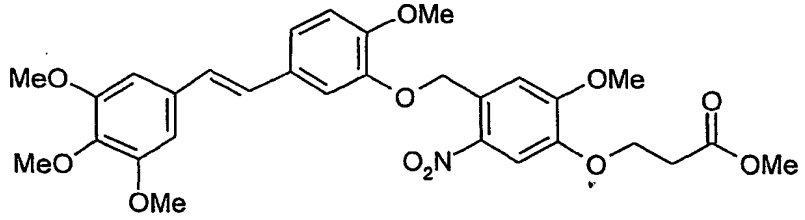


***Z*-11**

In a foil wrapped flask a suspension of the methyl ester ***Z*-8** (250 mg, 0.42 mmol) in 1M aqueous NaOH (0.84 ml, 0.84 mmol) was prepared. The yellow suspension was then heated at reflux for 40 mins, after which time water (5 cm^3) was added. The resulting orange solution was then acidified to pH 1 using conc. HCl and the subsequent yellow precipitate was filtered. The precipitate was purified by

recrystallisation from ethanol, which provided the desired compound as a pale yellow solid (214 mg, 81%). m.p. 138°C; Found C, 61.6; H, 5.4; N, 2.3; $C_{30}H_{33}NO_{11}$ requires C, 61.7; H, 5.7; N, 2.4%; R_f 0.10 (SiO_2, hexane:EtOAc 1:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3500-3100 (br), 3000-2800 (m), 1740 (s), 1610, 1580, 1520, 1330, 1280, 1220, 1130, 880; δ_H (300 MHz, $CDCl_3$) 2.21 (2H, q, *J* 6.5 Hz, CH_2), 2.63 (2H, t, *J* 6.5 Hz, CH_2), 3.67 [6H, s, $(OCH_3)_2$), 3.81 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 4.16 (2H, t, *J* 6.5 Hz, CH_2) , 5.40 (2H, s, CH_2), 6.43 (1H, d, *J* 12.9 Hz, olefinic CH), 6.47 (2H, s, H-2''' and H-6'''), 6.48 (1H, d, *J* 12.9 Hz, olefinic H), 6.81 (1H, d, *J* 7.9 Hz, H-3''), 6.91-6.95 (2H, m, H-4'' and H-6''), 7.45 (1H, s, H-3'), 7.74 (1H, s, H-6') ppm; δ_C (100 MHz, $CDCl_3$) 24.0 (CH_2), 30.1 (CH_2), 55.9 (OCH_3), 56.0 (OCH_3), 56.2 (OCH_3), 60.9 (OCH_3), 68.0 (CH_2), 68.4 (CH_2), 105.9 (CH), 109.4 (CH), 109.7 (CH), 111.6 (CH), 115.6 (CH), 123.1 (CH), 129.2 (CH), 129.3 (CH), 129.7, 130.3, 132.7, 137.2, 138.8, 146.9, 147.3, 149.0, 152.9, 154.3, 173.5 ($\underline{C}OOCH_3$) ppm; *m/z* (FAB) 583 [($M^{+\cdot}$), 100%].

Methyl 4-[2'-methoxy-4'-({2''-methoxy-5''-[(*E*)-2'''-3''',4''',5'''-trimethoxyphenyl)ethenyl]phenoxy}-methyl)-5'-nitrophenoxy]butanoate *E*-8

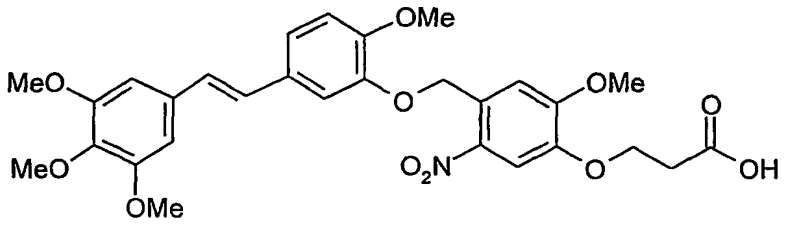


***E*-8**

To a suspension of methyl-4-[4'-(bromomethyl)-2'-methoxy-5'-nitrophenoxy] butanoate 7 (400 mg, 1.1 mmol) and *trans*-CA-4 (280 mg, 0.89 mmol) in anhydrous methanol (10 cm^3) was added K'OBu (148 mg, 1.32 mmol). The resulting yellow mixture was stirred at r.t.. for 30 mins after which time a yellow precipitate had formed. The precipitate was filtered and recrystallised from methanol furnishing the desired compound ***E*-8** as a pale yellow solid (367 mg, 73%). m.p. 142°C; Found C, 65.5; H, 6.4; N, 2.4; $C_{31}H_{35}NO_{11}$ requires C, 65.6; H, 6.2; N, 2.5%; R_f 0.44 (SiO_2, hexane:EtOAc 1:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3000-2800 (m), 1730 (s), 1610, 1580, 1520, 1320, 1280, 1220, 1130, 880; λ_{max}(MeCN)/nm 219 (ε 36612), 243 (ε 33655) and

329 (ε 41112); δ_H (300 MHz, $CDCl_3$) 2.20 (2H, p, *J* 6.8 Hz, CH_2), 2.57 (2H, t, *J* 6.8 Hz, CH_2), 3.70 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.92 [6H, s, $(OCH_3)_2$], 3.93 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.41 (2H, t, *J* 6.8 Hz, CH_2) , 5.61 (2H, s, CH_2), 6.73 (2H, s, H-2''' and H-6'''), 6.87 (1H, d, *J* 16.2 Hz, olefinic H), 6.94 (1H, s, H-3''), 6.95 (1H, d, J_H 16.2 Hz, olefinic CH), 7.11-7.14 (2H, m, H-4'' and H-6''), 7.55 (1H, s, H-3'), 7.77 (1H, s, H-6') ppm; δ_C (100 MHz, $CDCl_3$) 24.4 (CH_2), 30.4 (CH_2), 51.7 (OCH_3), 56.1 [$(OCH_3)_2$], 56.2 (OCH_3), 56.3 (OCH_3), 61.0 (OCH_3), 68.4 (2xCH_2), 103.3 (CH), 109.3 (CH), 109.7 (CH), 112.0 (2xCH), 120.7 (CH), 127.2 (CH), 127.5 (CH), 129.9, 130.7, 133.1, 137.8, 138.8, 147.0, 147.8, 149.5, 153.4, 154.5, 173.3 (<u>C</u>$OOCH_3$) ppm; *m/z* (FAB) 597 [($M^{+\cdot}$), 25%].

4-[2'-methoxy-4'-({2''-methoxy-5''-[(*E*)-2'''-3''',4''',5'''-trimethoxyphenyl)ethenyl]phenoxy}methyl)-5'-nitrophenoxy]butanoic acid *E*-11

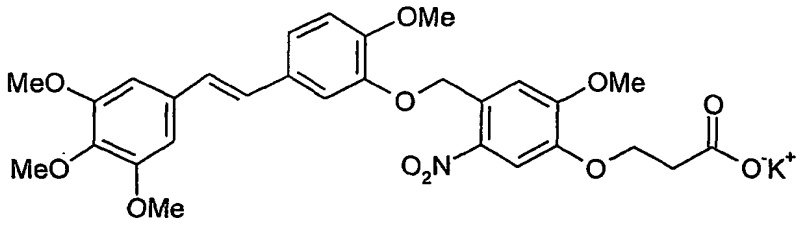


***E*-11**

In a foil wrapped flask a suspension of the methyl ester ***E*-8** (250 mg, 0.42 mmol) in 1M aqueous NaOH (0.84 ml, 0.84 mmol) was prepared. The yellow suspension was then heated at reflux for 40 mins, after which time water (5 cm^3) was added. The resulting orange solution was then acidified to pH 1 using conc. HCl and the subsequent yellow precipitate was filtered. The precipitate was purified by recrystallisation from ethanol, which provided the desired compound as a pale yellow solid (234 mg, 97%). m.p. 177°C; Found C, 62.0; H, 5.8; N, 2.5; $C_{30}H_{33}NO_{11}$ requires C, 61.7; H, 5.7; N, 2.4%; R_f 0.15 (SiO_2, hexane:EtOAc 1:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3500-3100 (br), 3000-2800 (m), 1710 (m), 1610, 1580, 1520, 1330, 1280, 1220, 1130; δ_H (300 MHz, $CDCl_3$) 2.25 (2H, q, *J* 6.7 Hz, CH_2), 2.66 (2H, t, *J* 6.7 Hz, CH_2), 3.90 (3H, s, OCH_3), 3.95 (3H, s, 2 x OCH_3), 3.96 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.19 (2H, t, *J* 6.7 Hz, CH_2) , 5.64 (2H, s, CH_2), 6.76 (2H, s, H-2''' and H-6'''), 6.93-6.98 (3H, m), 7.14-7.16, (2H, m, H-4'' and H-6''), 7.59 (1H, s, H-3'), 7.82 (1H, s, H-6')

ppm; δ_C (100 MHz, $CDCl_3$) 24.0 (CH_2), 30.0 (CH_2), 56.1 (2 x OCH_3), 56.2 (OCH_3), 56.3 (OCH_3), 61.0 (OCH_3), 68.1 (CH_2), 68.2 (CH_2), 103.3 (2 x CH), 109.3 (CH), 109.8 (CH), 112.0 (CH), 120.7 (CH), 127.2 (CH), 127.5 (CH), 130.1, 130.7, 133.1, 137.9 139.2, 146.8, 147.8, 149.5, 153.4, 154.8, 177.4 (COOH) ppm; *m/z* (FAB) 583 [($M^{+\cdot}$), 70%].

4-[2'-methoxy-4'-({2''-methoxy-5''-[(*E*)-2'''- 3''',4''',5'''-trimethoxyphenyl)ethenyl]phenoxy}methyl)-5'-nitrophenoxy]butanoic acid(potassium salt) *E*-12



***E*-12**

To a suspension of the butanoic acid ***E*-11** (250 mg, 0.43 mmol) in methanol (3 cm^3) was added K'OBu [0.43 cm^3, 0.43 mmol, (1 M methanolic solution)]. The resulting clear yellow solution was stirred at r.t. for 5 minutes before being concentrated *in vacuo* to provide a pale brown solid (0.26 mg, 97%).

Example 8: Photochemical cleavage and isomerisation

Methyl 4-[2'-methoxy-4'-({2''-methoxy-5''-[(*E*)-2'''- 3''',4''',5'''-trimethoxyphenyl)ethenyl]phenoxy}methyl)-5'-nitrophenoxy]butanoate, *E*-8

In a 400 cm^3 photochemical reaction vessel, N_2 was bubbled through distilled benzene (300 cm^3) for 30 mins. Following the degassing procedure, compound ***E*-12** (300 mg, 0.5 mmol) was added to the benzene and allowed to dissolve. The colourless solution was then irradiated using a 400W medium pressure Hg lamp for a total of 20 mins. Aliquots were removed from the reaction vessel at specific time points throughout the 20 mins (0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 mins). Two aliquots were removed from the reaction vessel at each time point, one sample (0.5 cm^3) was used for HPLC analysis (cyanopropyl column; mobile phase 95:5 hexane:IPA; flow rate 0.5 ml min^{-1}; lamp 245 nm), whilst the other sample (10 cm^3)

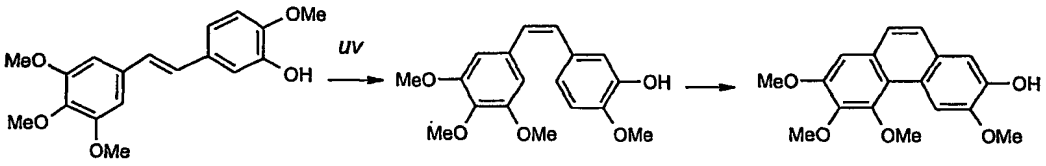
was concentrated *in vacuo* and used to determine the IC_{50} at that time point. After irradiation for 20 mins the reaction mixture was yellow/orange in colour.

The following compound retention times were observed:

Trans-CA-4	23.35 mins
Cis-CA-4	11.07 mins
Coupled *trans*-CA-4 *E*-8	43.89 mins
Coupled *cis*-CA-4 *E*-8	26.42 mins

Example 9: Photochemical isomerization of combrestatatin A-4 and derivatives

We detail the study of the *trans* to *cis* isomerisation of CA-4 herein. A solution of *trans*-CA-4 (200 mg, 0.63 mmol) in freshly distilled and degassed benzene (400 cm^3), prepared by bubbling through Ar for 2 hours prior to use, was irradiated using a 400 W medium pressure Hg lamp for a total of 20 mins. Aliquots were removed at regular intervals for GC analysis (180-280 °C at 5 °C min^{-1}; pressure program 195-225 kPa at 1.5 kPa min^{-1}; on a SE54 column).



***Trans*-CA-4** ***Cis*-CA-4** **Phenanthrene**

The results of GC analysis of the mixture are summarized in Table 3. Table 4 shows the retention times, by GC for the individual components of the reaction mixture.

Table 3:

Time (mins)	% *trans-stilbene*	% *cis-stilbene*	% phenanthrene
0	100	0	0
0.5	100	0	0
1.5	100	0	0
3.0	87	13	0
5.0	69	31	0
6.5	55	45	0
8.0	53	47	0
10.0	44	53	0
12.5	32	68	0
15.0	29	71	0
17.5	22	74	4
20.0	23	73	4

Table 4:

Component	Retention time (mins)*
trans-CA-4	20.32
cis-CA-4	15.42
CA-4 phenanthrene derivative **1**	19.00

* G.C. conditions; 180-280 °C @ 5 °C min^{-1}, 195-225 kPa @ 1.5 kPa min^{-1}, SE 54 column.

Table 5 shows the outcome of photoisomerisation of *trans*-CA-4 (99.7% *E*) and the corresponding IC_{50} values. The cytotoxicity (IC_{50} value) of the reaction mixture at each time point is shown in Table 3, showing an impressive increase in the cytotoxicity of the mixture over time.

The most exciting observation is that the IC_{50} at time zero is 4000 nM and after just half a minute it has decreased by more than 10 fold. This illustrates that the isomerization is rapid and also highlights just how potent *cis*-CA-4 is compared with *trans*-CA-4 since just 2.0% of *cis*-CA-4 present results in such a dramatic increase in cytotoxicity. The thousand-fold increase in activity obtained after six minutes, clearly shows that the process displays great potential.

Table 5:

Time (mins)	% *trans*	% *cis*	% phenanthrene	IC_{50} (K562) (nM)
0	99.7	0.3	0	4000
0.5	98.0	2.0	0	310
1.0	96.1	3.9	0	530
1.5	93.7	6.3	0	50
2.0	92.7	7.3	0	20
3.0	74.3	25.7	0	12
4.0	62.3	37.3	0	9
5.0	52.1	47.9	0	10
7.5	40.2	56.6	1.4	3
10.0	34.0	63.1	2.9	6
12.5	32.2	64.4	3.4	4
15.0	31.7	64.6	3.7	1
17.5	30.8	65.1	4.1	3
20.0	30.6	65.7	3.7	2

Previously, the isomerisation study had been performed *ex situ*, *i.e.* in a photochemical reactor. However, it was necessary to illustrate the potential of the process as a real therapy by repeating the isomerizaton in the presence of the cancer cells *i.e. in situ*. Therefore, K562 cells were dosed with a known concentration of *trans*-CA-4, the resulting solutions were then irradiated using an ultra violet lamp consisting of 2 x 7 W ultra violet tubes for given lengths of time. Following irradiation, the cells were incubated in the normal way and the IC_{50} values were determined. A series of control experiments were performed simultaneously to verify that the results obtained (illustrated in Table 6) were in fact due to the isomerisation process occurring and not due to the effect of the ultraviolet radiation. Table 6 shows that the same pattern of results is obtained. However, the decrease in the IC_{50} value is now more rapid.

The experiment convincingly supports the results derived from the *ex situ* study and we are able to show that the *trans* to *cis* isomerisation is occurring in both circumstances, and more importantly is causing a rapid and significant increase in the cytotoxicity of the system. Due to the speed with which the process was occurring the experiment was repeated and monitored every 5 seconds in the first minute of

irradiation. The results are shown in Table 7. It can be seen that after just 5 seconds there is a greater than 15 fold reduction in the IC_{50} and after a further 40 seconds a single nanomolar figure IC_{50} value is reached.

Table 6:

Time/mins	IC_{50} K562 nM
0	1200
1	3.6
2	2.9
3	1.7
4	4.4
5	2.7
6	2.9
7	1.9
8	3.0
9	2.5
10	2.6
12.5	2.4
15.0	2.0
17.5	1.7
20.0	2.7

Table 7:

Time/secs	IC_{50} K562 nM
0	580
5	35
10	40
15	20
20	18
25	17
30	15
35	11
40	11
45	9
50	9
55	9
60	8

Example 10: Synthesis of *trans*-CA-4 bearing a photocleavable group

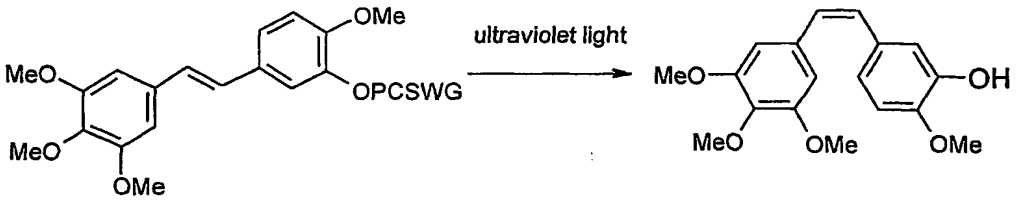
We next demonstrate the potential use of light to trigger the release of CA-4 from a variety of non-toxic CA-4 derivatives. By way of example, two types of reaction were employed:

(1) Release of CA-4 from a *trans*-CA-4 derivative with *E* to Z isomerization.

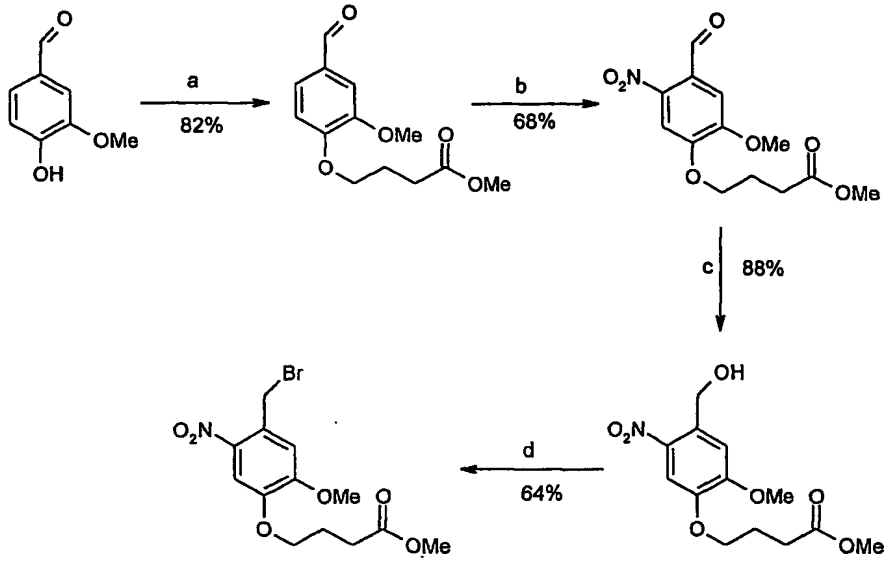
(2) Release of CA-4 from a photolabile *cis*-CA-4 derivative.

As in some applications in the prior art, the insolubility of CA-4 in water results in clinical difficulties, it would be advantageous to utilize a photocleavable group to impart water solubility to the system.

We first considered the fate of a *trans*-CA-4 derivative (**2**) bearing a photocleavable water solubilising (PCWS) group (see the scheme below). When irradiated with ultraviolet light, two events can occur; (i) the PCWS group can be cleaved and (ii) *trans* to *cis* isomerisation can take place, (but perhaps not in that order). The overall effect of these transformations should be a dramatic increase in cytotoxicity.

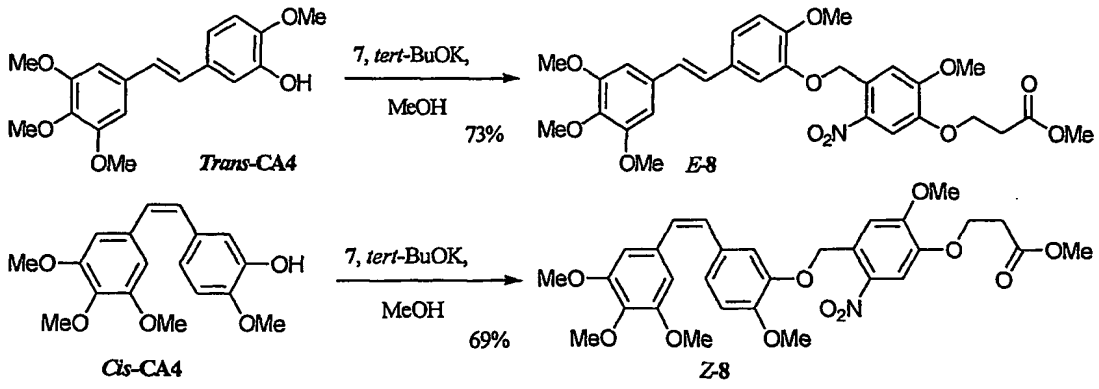


It became apparent that whilst there are examples of the use of photocleavable groups in medical applications there are no examples of their use in this context. To demonstrate the validity of this approach we used an *ortho*-nitrobenzyl derivative as a prototypical photocleavable group. A vanillin based compound, developed by Holmes *et al.* (C. Holmes, *J. Org. Chem.*, 1997, 62, 2370.) as a solid phase linker, was used as a template for our photocleavable group. The synthetic strategy to the coupling agent **7** is outlined in the following scheme.



a) Methyl-4-bromobutyrate, K_2CO_3, DMF; b) HNO_3, DCM, -5°C; c)$NaBH_4$, THF, d) PBr_3, DCM.

The next step was to couple the benzyl bromide **7** to *trans*-CA-4. The reaction gave the desired product ***E*-8** in a 73% yield, as illustrated below. ***E*-8** precipitated out of solution as the reaction to place and was subsequently filtered and purified by recrystallisation. The coupling reaction was repeated using *cis*-CA-4 as the substrate–the reaction proceeded in a 69% yield.



As with the *trans* to *cis* photoinduced isomerisation study coupled *trans*-CA-4 was dissolved in benzene and the resulting solution was irradiated for a total of 20 minutes with aliquots removed at given time points to determine the isomeric ratios and the IC_{50} value. The results obtained are detailed in Table 8.

Table 8:

Time/mins	% coupled *trans*-CA-4 (*E*-8)	% *trans*-CA-4	% *cis*-CA-4	IC_{50} K562 (nM)
0	100	-	-	>150000
0.5	93	7	-	14580
1.0	88	8	4	450
1.5	80	12	8	90
20.	72	19	9	59
3.0	62	27	11	14
4.0	61	31	8	12
5.0	53	37	10	8
7.5	42	45	13	4
10.0	34	47	19	6
12.5	20	35	45	2
15.0	Unable to calculate*			2
17.5	Unable to calculate*			4
20.0	Unable to calculate*			3

The results show that the photocleavage of *trans*-CA-4 is the first event to occur, followed soon after by the isomerisation of *trans*-CA-4 to *cis*-CA-4. This process is accompanied by an even more dramatic increase in the cytotoxicity than was seen previously, this is largely because ***E*-8** is much less cytotoxic than *trans*-CA-4, in fact it is 5 times less cytotoxic. This is highly beneficial if ***E*-8** were to be used as a CA-4 prodrug. These results also highlight the speed with which the process occurs. After just 5 minutes exposure to ultra violet light the IC_{50} value has fallen to single nanomolar figures, with slightly more than 50% of the coupled starting material remaining and some 10% *cis*-CA-4 present.

Once again it was desirable to investigate the effect of the coupled *trans*-CA-4, ***E*-8** upon irradiation in the presence of cancer cells. Therefore, K562 cells were dosed with a known concentration of coupled *trans*-CA-4 ***E*-8** and subsequently exposed to ultra violet radiation for specific lengths of time and the IC_{50} value determined. As with the previous *in situ* experiment a series of control experiments were performed simultaneously to verify that any positive results obtained were due to the effect of ultra violet light on the drug candidate and not its effect on the cells. These consisted

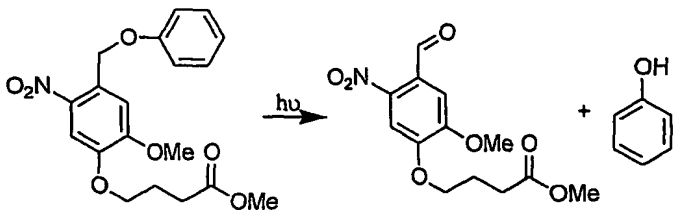
of i) exposing K562 cells only (*i.e.* cells which were not dosed with any potential drug candidate) to ultra violet light, ii) preventing K562 cells dosed with drug candidate from being exposed to ultra violet light, and iii) preventing K562 cells only (*i.e.* cells which were not dosed with any potential drug candidate) from being exposed to ultra violet light. In all three control experiments no cytotoxic effect was observed. The results of the main experiment are illustrated in Table 9. It is apparent that the same general trend, with respect to the IC_{50} values is seen in both the *ex situ* and the *in situ* experiments.

Table 9:

Time/Mins	IC_{50} K562 (nm)
0	>100000
0.5	1540
1	450
1.5	250
2	130
3	40
4	11
5	9
7.5	5
10	2
12.5	3
15	3
17.5	2
20	2

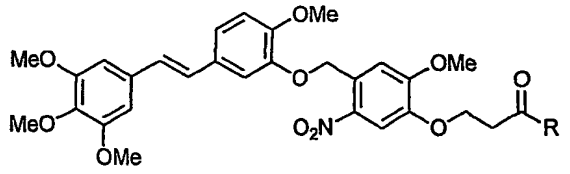
We needed to demonstrate that the photo by-product, the nitrosobenzaldehyde, **10** did not possess any cytotoxicity and therefore was not contributing to the results obtained for the photoactivation of coupled *trans*-CA-4. The synthesis of the nitroso compound, **10** was achieved by exposing compound **9** to ultra violet light for 10

minutes and the desired compound was isolated from the resulting reaction mixture *via* column chromatography (see the scheme below). The compound **10** lacked significant cytotoxicity.



9 **10**

Having convincingly established the feasibility of prodrug photoactivation, the final objective was to render the coupled *trans*-CA-4 compound, ***E*-8** water-soluble. A water soluble prodrug of CA-4 would clearly have clinical potential. Therefore, a water-soluble derivative was synthesised in an attempt to overcome this problem. The water-soluble derivative was prepared from the methyl ester, ***E*-8** by a simple hydrolysis reaction using NaOH in ethanol. However, due to purification problems the carboxylic acid, ***E*-11** was isolated and subsequently deprotonated with K'OBu, the water-soluble salt was isolated using ion-exchange chromatography.



E-8 R = OMe —a, 78%→ *E*-11 R = OH —b, 80%→ *E*-12 R = OK

a. (i) NaOH, MeOH; (ii) HCl; b. *tert*-BuOK, H_2O

The *in situ* study of the water-soluble derivative of *trans*-CA-4 was performed in the same manner as those described previously and the results can be seen in Table 10. The results obtained suggest that the water-soluble derivative, ***E*-12** behaves in a similar fashion as the methyl ester derivative, ***E*-8** when irradiated with ultra violet light in the presence of cancer cells. The IC_{50} values obtained are similar to those obtained for the *in situ* study of the methyl ester derivative, ***E*-8**.

Table 10:

Time/Mins	IC_{50} K562 (Nm)
0	>50000
0.5	1010
1	410
1.5	300
2	160
3	50
4	58
5	46
7.5	22
10	16
12.5	12
15	10
17.5	9
20	8

Example 11: Further Biological Experiments

The effect of compounds 97-64H and 97-96 (quinone compound) was tested on H460 human lung xenograft grown subcutaneously by injecting equitoxic doses of the compounds (0.75 x MTD). Twenty four hours following injection (i.p.) both compounds caused extensive damage to the tumours which was consistent with necrosis caused by destruction of vasculature.

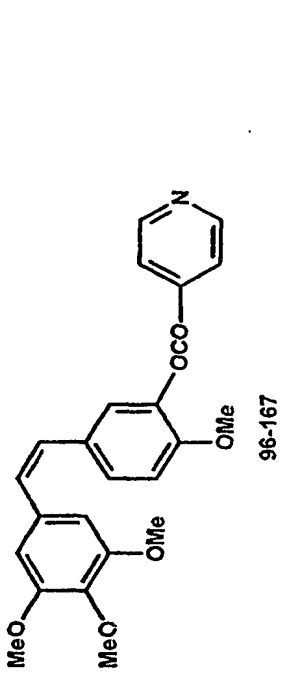
The results from testing compounds of the invention in cell based assays are reported in Tables 12 to 27 below.

The references cited herein are incorporated by reference.

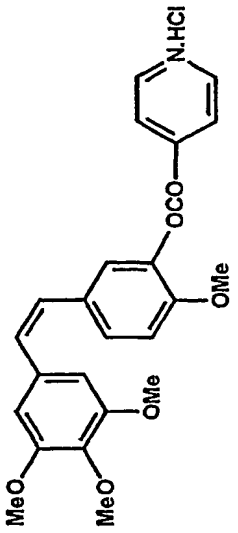
Compound	P388 $1C_{50}$ (μM)	A2780 $1C_{50}$ (μM)	H460 $1C_{50}$ (μM)	K562 (μM)	Tub Ass $1C_{50}$ (μM)	Colchicine % Inhibition	HUVEC $1C_{50}$ (μM)	Permeability
Comb A-4 (1)	0.0026	0.00072	1.51		2.4	12	0.001	7.1, 6.9
97-64H	0.000489	0.00366			<1.25, 12.5	79, 70	.0024	5.8
97-64L	0.171	0.113			>100	43	NT	1.0
97-65	0.18	0.095			100	NT	0.35	3.4
96-188	0.32	.081			NT	NT		
96-167	0.0008	0.00037			9	3.8	0.04	5.5
97-07	0.0023	0.00147	2.78		10	6.6, 12.3		
97-13H	0.0326	0.0179	.032-.035		>100	84.2	0.045	4.3, 3.2
98-21	.000935	.00066			<1.25	1.4	0.0095	4.5
97-96	0.57	0.19	.038		<1.25	82, 24	0.23, 0.22	2.2
98-23	2.18	2.15	4.37		NT	NT		
98-35H	.00328	.00144			6.25	5	0.016	7.4
98-29	0.45	0.38			>100	53	0.43	3.9
99-03H	0.19	0.12		0.06	60			
00-82		.06		0.05	7.2	39.5		
00-105				0.02	>100	3.6		
17a				0.2				
17b				6				
19				0.04	1.8			
20	7			1.8				
23a				0.1				
23b				0.79				
27				9				
28				0.021	1.5			
31a				0.018	7.5			
32a				0.044	18			
45				0.12	1.8			

NT=NOT TESTED; 'H' COMPOUNDS ARE *CIS*, 'L' COMPOUNDS ARE *TRANS*. Stilbenes synthesised by either *Wittig* or 2-step synthesis routes. Quinone synthesised as in Section 9.

TABLE 11



96-167



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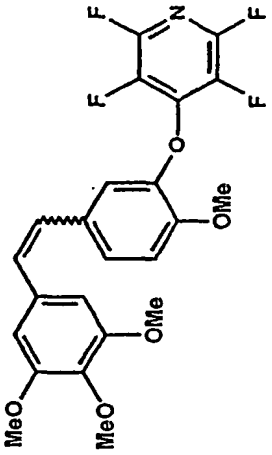

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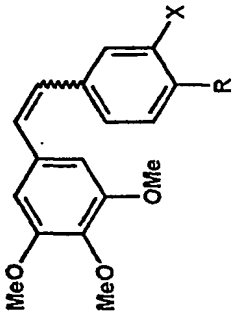
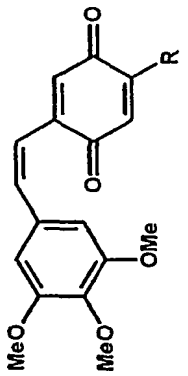
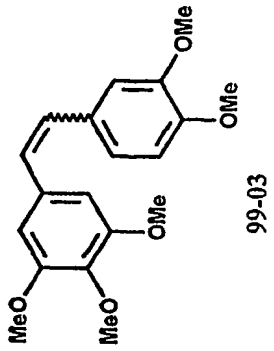


97-64 R = OMe, X = F
97-65 R = NMe_2.HCl, X= H
96-188 R = CHO, X = H
98-21 R = Me, X = OH
98-35 R = Me, X = F

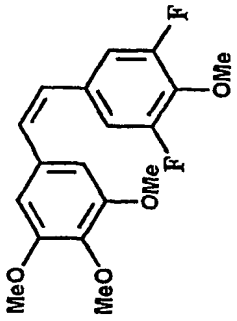


97-96 R = OMe

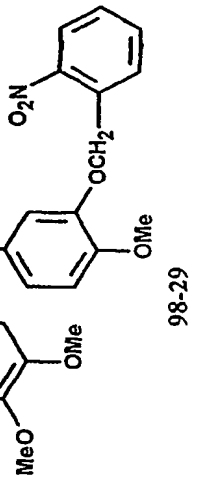
98-23 R = Me



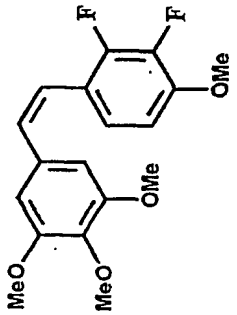
99-03



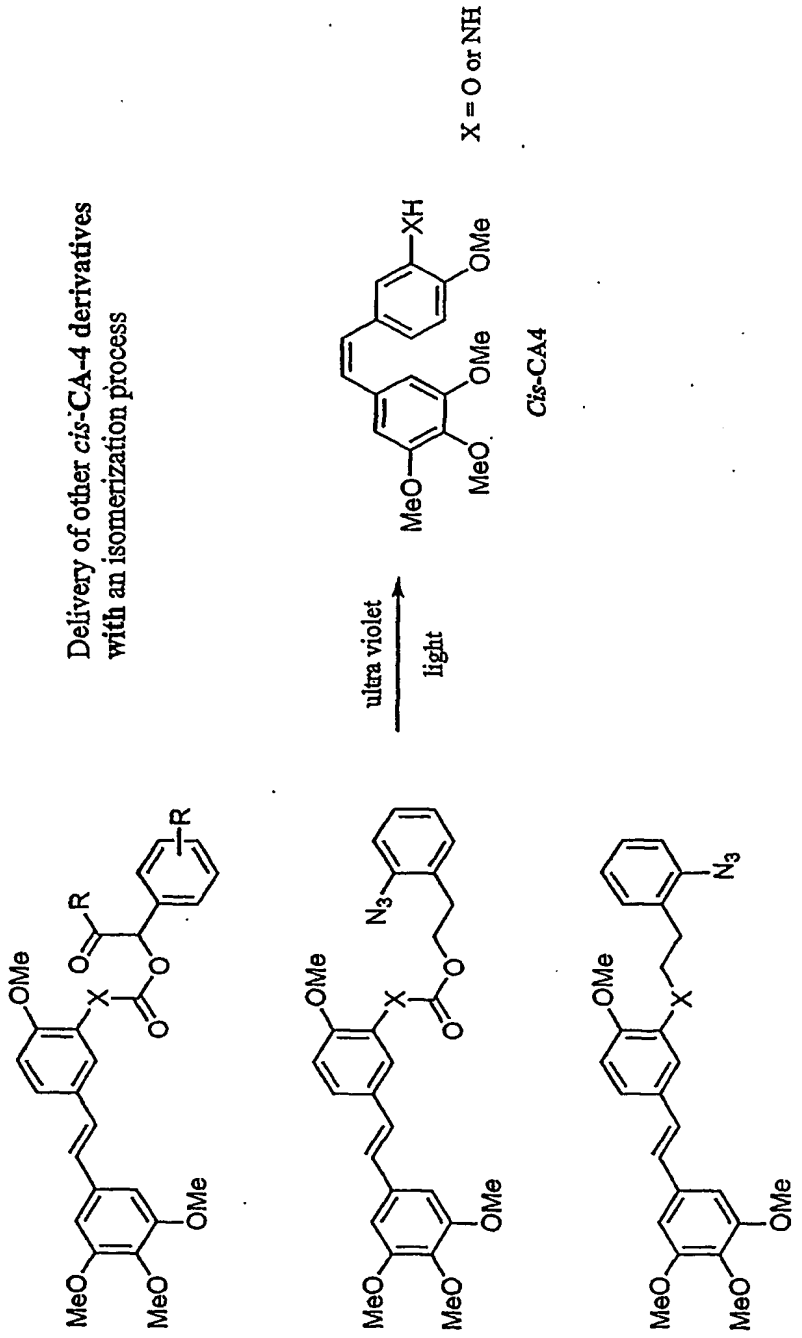
00-82



98-29



00-105


Delivery of other cis-CA-4 derivatives
with an isomerization process
ultra violet
light
X = O or NH
Cis-CA4
OMe
MeO
MeO
OMe
XH
N3
R
O
X

Other derivatives that will deliver *cis*-CA-4 with an isomerization process

Other derivatives that will deliver *cis*-CA-4 without an isomerization process

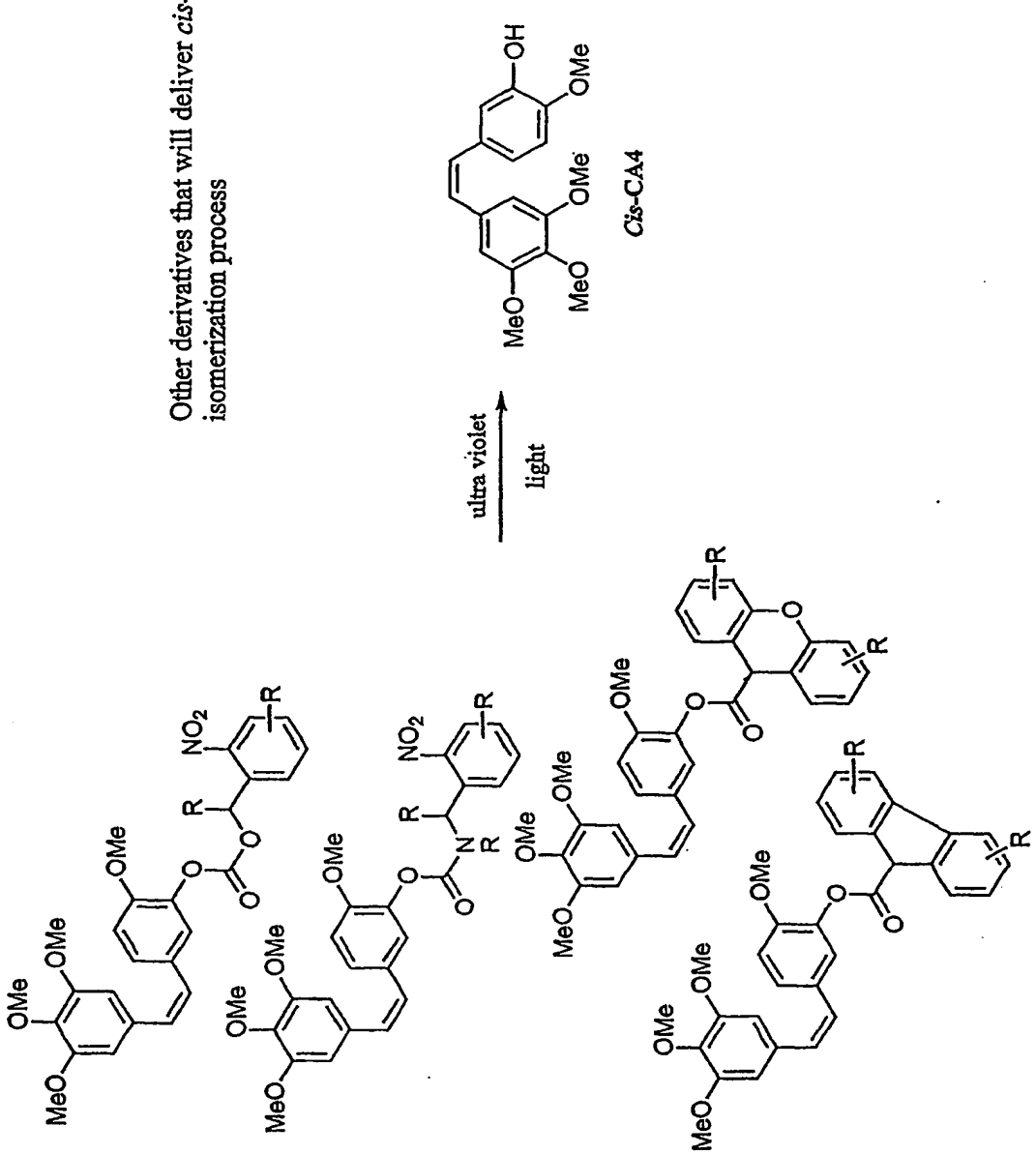
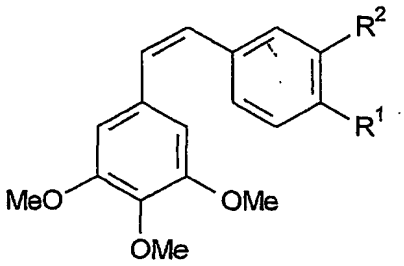


Table 12: Biological activity of stilbenes with 3,4,5-trimethoxy substitution on the A ring

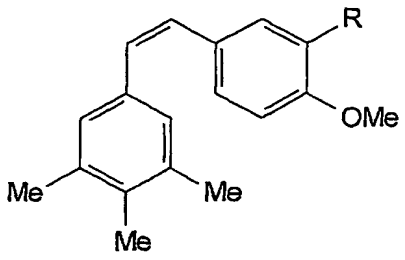


Compound number	R^2	R^1	Config	MTT (P388) IC_{50} (μM)	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
CA4 (15)	**OH**	**OMe**	***Z***	**0.003**	**0.001**	**0.175**	**3**
32	H	OMe	*Z*	ND	0.004	0.2	5.5
90	F	OMe	*Z*	ND	0.01	0.085	2.8
93	Br	OMe	*Z*	ND	0.001	0.4	10
96			*E*	ND	7.83	>10	>25
101	OH	H	*Z*	0.3	0.14	>10	>25
102			*E*	34	22	>10	>25
103	Me	Me	*Z*	0.1	0.04	2	>25
104			*E*	20	35.8	>10	>25

Table 13: Growth inhibition studies using HUVEC

Drug	HUVEC IC_{50} (μM)
CA4 (15)	**0.0026**
90	0.004
32	0.006
93	0.067

Table 14: Biological activity of stilbenes with 3,4,5-trimethyl substitution on the A ring



Compound number	R	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
Combretastatin A-4 (15)		**0.001**	**0.175**	**3**
117	H	0.31	0.650	>25
120	F	0.14	0.700	10
133	OH	0.020	0.120	10

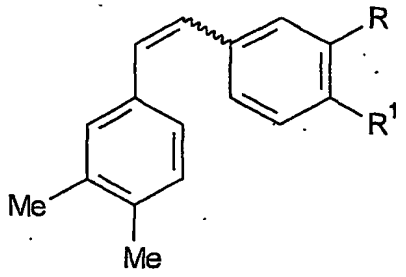
Table 15: Growth inhibition studies using HUVECs

Drug	HUVEC IC_{50} (μM)
117	>1
120	0.46
133	0.05

Table 16: K562 cell cycle analysis

Drug	% of cells with DNA content <2n	% of cells with DNA content = 2n or >2n		
		% of cells in G_0-G_1	% of cells in S phase	% of cells in G_2-M
117	15	3	10	87
120	23	6	17	77
133	6	3	6	91

Table 17: Biological activity of stilbenes with 3,4-dimethyl substitution on the A-ring

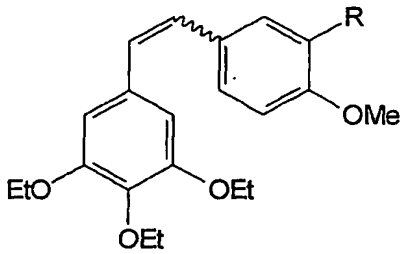


Compound number	R	R^1	Config	MTT (P388) IC_{50} (μM)	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
Combretastatin A-4 (15)			*Z*	**0.003**	**0.001**	**0.175**	**3**
137	H	Me	*Z*	2	3.4	>10	>25
138			*E*	17	>50	>10	>25
139	NO_2	OMe	*Z*	20	1.8	>10	>25
140			*E*	>50	24	>10	>25
141	H	OMe	*Z*	4.9	1.6	>10	>25
142			*E*	20.5	>50	>10	>25
143	Br	OMe	*Z*	2.5	1.15	>10	>25
144			*E*	>50	>50	>10	>25
146	OH	OMe	*Z*	0.4	0.07	3.09	12.5
147	F	OMe	*Z*	1.7	0.09	6.57	>25
148			*E*	36	2.5	>10	>25
149	F	Me	*Z*	7.2	0.39	>10	>25
150			*E*	40	>50	>10	>25
151	NO_2	Me	*Z*	9.7	1.5	>10	>25
152			*E*	>50	>50	>10	>25

Table 18: K562 cell cycle analysis

Drug	% of cells with DNA content <2n	% of cells with DNA content = 2n or >2n		
		% of cells in G_0-G_1	% of cells in S phase	% of cells in G_2-M
Combretastatin A-4 (15)	**7**	**3**	**6**	**92**
146	31	8	27	65
147	28	10	33	57

Table 19: Biological activity of stilbenes with 3,4,5-triethoxy substitution on the A-ring



Compound number	R	Config	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
Combretastatin A-4 (15)		*Z*	0.001	0.175	3
247	Br	*Z*	0.6	>10	>25
248		*E*	>50	>10	>25
249	H	*Z*	0.5	>10	>25
250		*E*	>50	>10	>25
251	F	*Z*	0.044	1.25	>25
252		*E*	>50	>10	>25
253	OH	*Z*	0.018	0.50	15.5
254		*E*	0.2	>10	>25
255	Br	*Z*	0.6	>10	>25
256		*E*	>50	>10	>25
257	Cl	*Z*	0.45	>10	>25
258		*E*	7	>10	>25

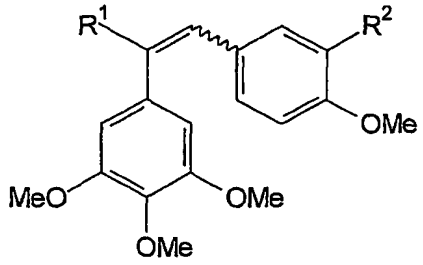
Table 20: Growth inhibition studies using HUVECs

drug	HUVEC IC_{50} (μM)
253	0.05
251	0.19

Table 21: K562 cell cycle analysis

Drug	% of cells with DNA content <2n	% of cells with DNA content = 2n or >2n		
		% of cells in G_0-G_1	% of cells in S phase	% of cells in G_2-M
253	4	3	6	92
251	24	7	18	75

Table 22: Biological activity of stilbenes with substitution on the olefinic bond



Compound number	R^1	R^2	Config	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
Combretastatin A-4 (15)			*Z*	**0.001**	**0.175**	**3**
208	Me	OTBDMS	*Z*	0.2	1.5	>25
209			*E*	6	>10	>25
210	Me	OH	*Z*	0.04	0.13	6
211			*E*	0.7	>10	>25
213	Me	H	*Z*	0.1	1.3	>25
214			*E*	0.8	>10	>25
217	Et	OTBDMS	*Z*	0.5	>10	>25
218			*E*	3.4	>10	>25
219	Et	OH	*Z*	0.12	0.13	>25
220			*E*	4	>10	>25
80	CO_2H	OH	*E*	>50	>10	>25
81	CO_2Me	OH	*E*	>50	>10	>25
82	CH_2OH	OH	*E*	>50	>10	>25

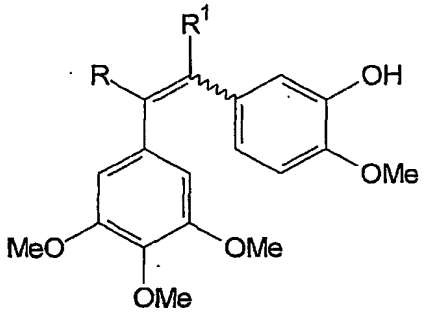
Table 23: Growth inhibition studies using HUVECs

Drug	HUVEC IC_{50} (μM)
210	0.09
213	0.35
219	0.22

Table 24: K562 cell cycle analysis on double bond substituted analogues

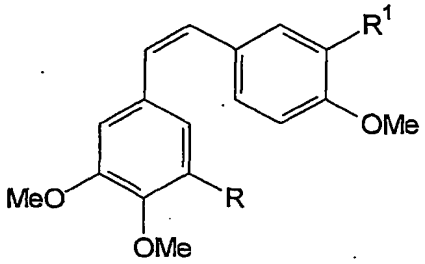
Drug	% of cells with DNA content <2n	% of cells with DNA content = 2n or >2n		
		% of cells in G_0-G_1	% of cells in S phase	% of cells in G_2-M
210	23	5	22	73
213	23	5	21	73
219	24	7	19	74

Table 25: Biological activity of stilbenes with substitution on the double bond



Compound number	Config	R	R^1	MTT (P388) IC_{50} (μM)	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
200	*E*	H	Me	7	2	9	>25
210	*Z*	Me	H	ND	0.04	0.13	6
211	*E*	Me	H	ND	0.7	>10	>25

Table 26: Biological activity of monofluoro prodrug precursors



Compound number	R	R^1	Config	MTT (K562) IC_{50} (μM)	MA IC_{50} (μM)	CD IC_{50} (μM)
Combretastatin A-4 (15)			*Z*	**0.001**	**0.175**	**3**
240	OTBDMS	F	*Z*	0.5	>10	>25
241			*E*	10	>10	>25
242	OH	F	*Z*	0.02	1.25	9
243			*E*	5	>10	>25
90	OMe	F	*Z*	0.01	0.085	2.8
18	OH	OH	*Z*	0.04[a]	4-5	22

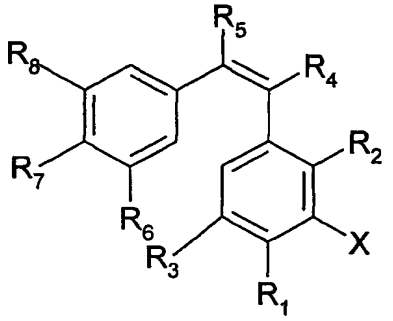
[a]L1210 murine leukaemia cell line

Table 27: K562 cell cycle analysis

Drug	% of cells with DNA content <2n	% of cells with DNA content = 2n or >2n		
		% of cells in G_0-G_1	% of cells in S phase	% of cells in G_2-M
242	29	7	22	71
90	16	5	16	79

Claims:

1. A compound represented by the structural formula:



wherein:

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

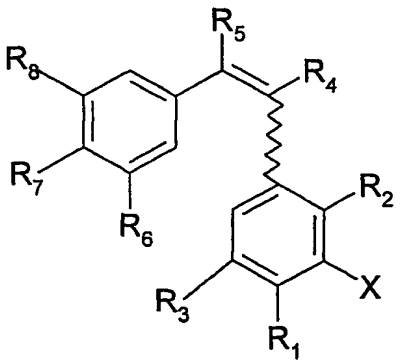
R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy;

or a salt or derivative thereof.

2. A compound represented by the structural formula:



wherein:

the zigzag line indicates that the compound can be *cis* or *trans*;

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', S, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy;

wherein at least one of the substituents R_4 and R_5 is an alkyl group.

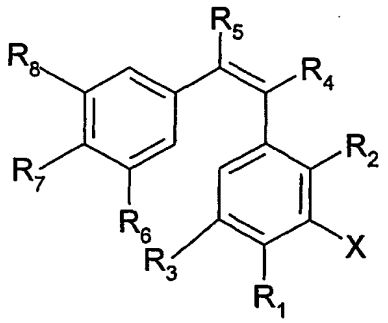
or a salt or derivative thereof.

3. The compound of claim 2 which is the *cis* or *Z*-isomer.

4. The compound of claim 2 which is the *trans* or *E*-isomer.

5. The compound of any one of claims 2 to 4, wherein the alkyl group R_4 and/or R_5 is a methyl or ethyl group.

6. A compound represented by the structural formula:



wherein:

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-

aryl, O-heteroaryl or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

wherein R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy such that at least one of these substituents is an alkyl group;

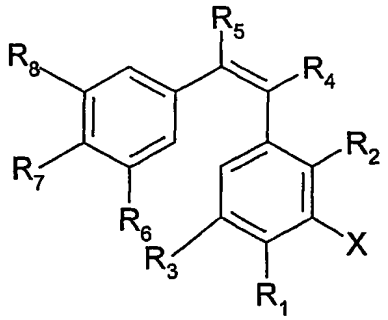
or a salt or derivative thereof.

7. The compound of claim 6, wherein two of the R_6, R_7 and R_8 groups are alkyl groups.

8. The compound of claim 6, wherein all three of the R_6, R_7 and R_8 groups are alkyl groups.

9. The compound of any one of claims 6 to 8, wherein the groups of the R_6, R_7 and R_8 groups which are alkyl groups are methyl, ethyl or propyl groups.

10. A compound represented by the structural formula:



wherein:

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl, or O-ester;

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR'' or CH_2CONHR''; and,

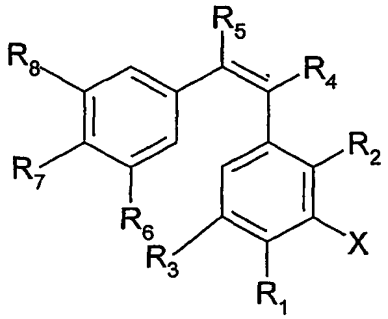
wherein R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy such that at least one of these substituents is an alkoxy group other than methoxy group;

or a salt or derivative thereof.

11. The compound of claim 10, wherein two of the R_6, R_7 and R_8 groups are alkoxy groups other than methoxy.

12. The compound of claim 10, wherein all three of the R_6, R_7 and R_8 groups are alkoxy groups other than methoxy.

13. A compound represented by the structural formula:



wherein:

R_1 is selected from alkyl, alkoxy, NH_2, NHR, NRR', SR, CF_3, CHO or halogen;

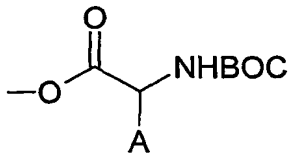
R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR'' or CH_2CONHR''; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy; and,

or a salt or derivative thereof;

wherein X is a group represented by:

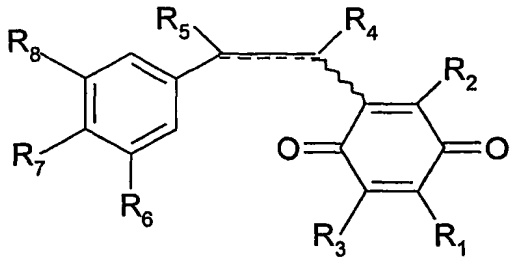


wherein BOC represents a t-butoxycarbonyl group and the A group is an amino acid side chain.

14. The compound of claim 13, wherein the BOC amino acid ester comprises Phe, Ile, Gly, Trp, Met, Leu, Ala, His, Pro, D-Met, D-Trp, or Tyr.

15. The compound of claim 13 or claim 14, wherein the amino acid is Phe, the A group is $-CH_2Ph$.

16. A compound represented by the structural formula:



wherein:

the dotted line indicates a single or double covalent bond and the zigzag line indicates that the compound can be *cis* or *trans*;

R_1, R_2 and R_3 are independently selected from hydrogen, alkyl, CHO, COR, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy; or

a salt or derivative thereof.

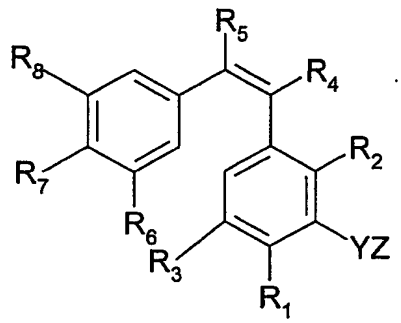
17. A composition comprising one or more of the compounds of any one of claims 1 to 16.

18. A compound of any one of claims 1 to 16 for use in a method of medical treatment.

19. Use of a compound of any one of claims 1 to 16 for the preparation of a medicament for the treatment of cancer or a condition characterised by abnormal proliferation of the vasculature.

20. The use of claim 19, wherein the condition characterised by abnormal proliferation of the vasculature is diabetic retinopathy, psoriasis or endometriosis.

21. A prodrug comprising a compound conjugated to a photocleavable group, wherein the prodrug is represented by the general formula:



wherein:

R_1 is selected from alkyl, CHO, alkoxy, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR'' or CH_2CONHR''; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkyoxy;

Y is selected from O, S, Se, NH; and,

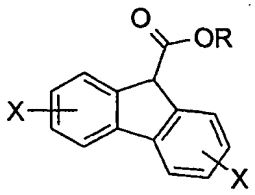
Z is a photocleavable group;

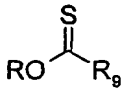
or a salt or derivative thereof.

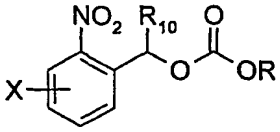
22. The prodrug of claim 21, wherein the compound conjugated to the photocleavable group is combretastatin or a derivative thereof.

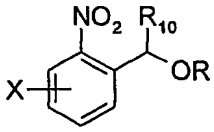
23. The prodrug of claim 22, wherein the combretastatin is *cis*-combretastatin A4.

24. The prodrug of any one of claims 21 to 23, wherein Z, the photocleavable group, is selected from:









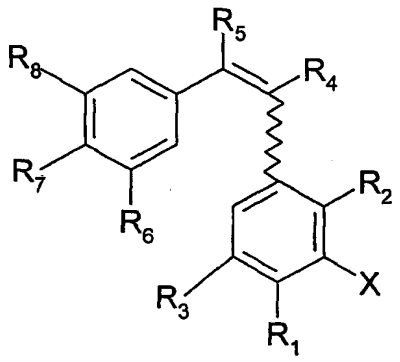
wherein R is the photoprotected group, R_9 and R_{10} are independently selected from alkyl, aryl or heteroaryl and X is any functional group.

25. The prodrug of any one of claims 21 to 24 for use in a method of medical treatment.

26. Use of the prodrug of any one of claims 21 to 24 for the preparation of a medicament for the treatment of cancer or a condition characterised by abnormal proliferation of the vasculature.

27. The use of claim 26, wherein the condition characterised by abnormal proliferation of the vasculature is diabetic retinopathy, psoriasis or endometriosis.

28. A process for isomerising a compound represented by the general formula:



wherein:

R_1 is selected from alkyl, alkoxy, CHO, NH_2, NHR, NRR', SR, CF_3 or halogen;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkoxy, hydroxyl, NH_2, NHR, NRR', SR, haloalkyl or halogen;

R_4 and R_5 are independently selected from hydrogen, alkyl, CH_2NHCOR" or CH_2CONHR"; and,

R_6, R_7 and R_8 are independently selected from hydrogen, alkyl or alkoxy;

X is selected from hydroxyl, nitro, amino, aryl, heteroaryl, alkyl, alkoxy, CHO, COR, halogen, haloalkyl, NH_2, NHR, NRR', SR, $CONH_2$, CONHR, CONHRR', O-aryl, O-heteroaryl, O-ester, or the group Y-Z as defined above;

or a salt or derivative thereof;

the process comprising exposing the compound to light so that it isomerises from the *E*-isomer to the *Z*-isomer.

Figure 1

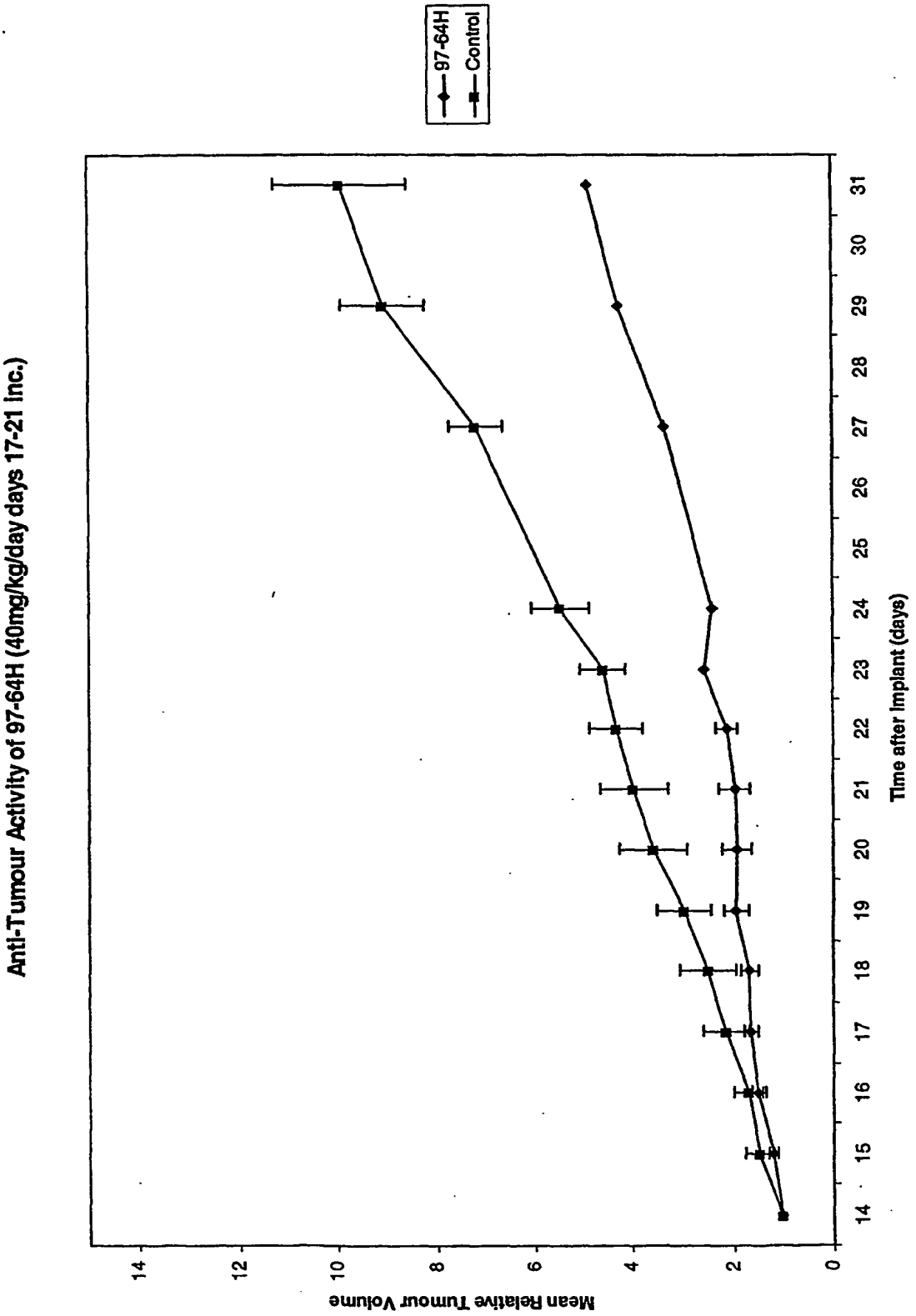
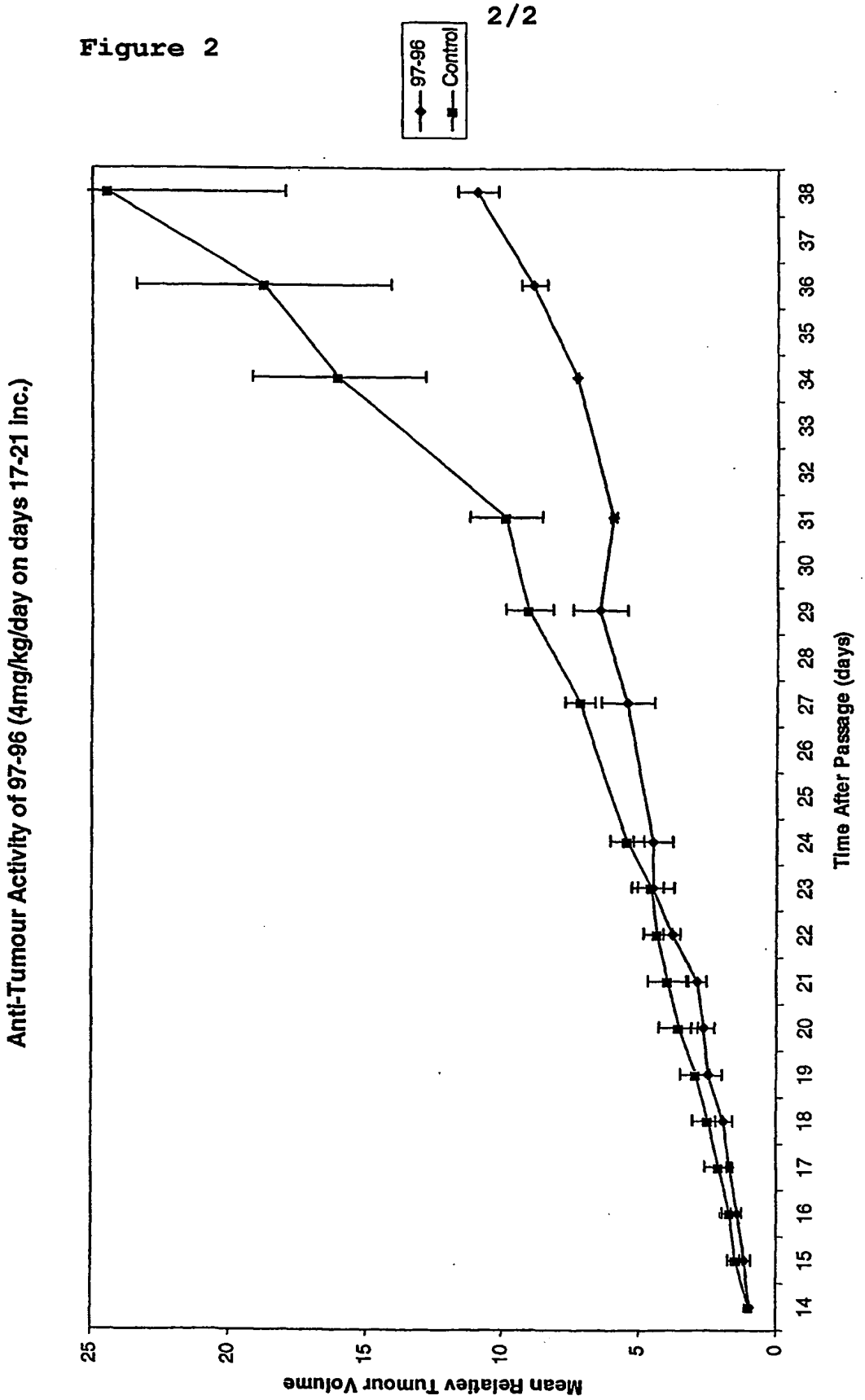

97-64H
Control
Anti-Tumour Activity of 97-64H (40mg/kg/day days 17-21 inc.)
Mean Relative Tumour Volume
Time after Implant (days)
0
2
4
6
8
10
12
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

Figure 2


97-96
Control
Anti-Tumour Activity of 97-96 (4mg/kg/day on days 17-21 inc.)
Mean Relative Tumour Volume
Time After Passage (days)
0
5
10
15
20
25
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
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36
37
38